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Total Bacterial Count and Presence of Escherichia coli in Raw Ground Beef Samples Obtained From Three Major Supermarkets in Bonaire, Dutch Caribbean

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Abstract

Background: There is no information on the microbiological quality of retailed ground beef in Bonaire, Dutch Caribbean. The objective of this study was to determine the total bacterial counts and detect the presence of *Escherichia coli* in samples of ground beef .sold in three major supermarkets in Bonaire.

Methods: A total of 36 samples of raw ground beef comprising 12 from each of the three supermarkets collected over a period of 4 weeks were examined. Dilutions of each sample were inoculated on three plates of nutrient agar by standard procedure. The plates were incubated at 37^0C/room temperature for 48 hours to determine the total bacterial count as colony forming units per gram (CFU/g). Additionally, 5 samples were used from each of the supermarket to detect the presence of *E. coli*.

Results: It was found that the mean bacterial count of CFU/g of ground beef samples from supermarket 1 was significantly higher (224,800) than that from the supermarkets 2 (5280) and 3 (4800). The mean *E. coli* count in the samples from supermarkets 1 and 2 was 52 and 9 CFU/g respectively.

Conclusion: This study is the first attempt to assess the overall microbiological quality of raw ground beef retailed in Bonaire by demonstrating significant differences in the means of total bacterial CFU/g, and counts of *E. coli* in samples from different supermarkets. This information can guide the producers of ground beef and the management of the supermarkets to recognize the good microbial quality of ground beef and to prevent bacterial contamination during handling.

Keywords: Raw Ground Beef; Bonaire; Total Bacterial Count, *Escherichia Coli*.

Introduction

Raw meats sold in supermarkets often contain *Salmonella*, *Escherichia coli, Staphylococus aureus*, and other bacteria [1]. These bacteria cause thousands of cases of illness, some of which result in hospitalizations and mortality each year, and their several strains have developed resistance to common antibiotics [2,3]. Cross contamination can also occur when safe food handling procedures are not followed. Therefore, the quality of the retailed raw meat is important for the health of the local population. Total bacterial counts (also called Aerobic Plate Counts or APC) and presence of *E. coli* in meats are used to help determine general hygiene, quality and safety of meat products [4-6]. These counts help determine the shelf life of meat. Lower initial bacterial counts are associated with increased shelf life, while higher initial bacterial counts lead to more rapid development of slime and other effects of meat spoilage [7]. Increased shelf life allows consumers more freedom in timing of meat purchase and meat preparation. While proper cooking destroys most of these bacteria, many people consume raw beef (as in steak tartar) or undercook or improperly cook the meat before eating. Thus it was considered worthwhile investigating the bacteriological quality of raw ground beef sold in supermarkets in Bonaire from the public health point of view.

Methods

Sample Collection

Separate samples of raw ground beef were collected (by purchase) from the regular chilled displays in the three largest supermarkets located on the island of Bonaire on each Monday, Wednesday

and Friday over a period of 4 weeks from November 11 to December 6, 2013. This comprised a total of 36 samples. Additional samples were obtained prior to November 11 for use in refining the total bacterial count method and preparing the Gram stained smears of the ground beef on microscopic slides. All samples were placed in an iced cooler and transported to laboratory for prompt processing.

Sample Processing and Total Bacterial Count Determination

From each sample of ground beef one gram was aseptically weighed on an electronic balance cleaned with an alcohol swab, and placed into a sterile test tube containing 10 mL of sterile distilled water. It was agitated for 2 minutes and 0.01 (equivalent to 0.01g) quantities of the suspension were delivered to each of the three prepared petri dishes of sterile nutrient agar (Oxoid), using a sterile inoculating loop. The inoculum was spread uniformly using a sterilized glass spreader. In a prior test, this quantity of meat was found to yield acceptable numbers of colony counts per dish (30-300). Inoculated petri dishes were incubated for 48 hours initially in both at Bonaire ambient room temperature (22^0C-30 0C) and at 37^o C in the Bonlab located a little faraway. Both incubation methods yielded similar results. Therefore, for ease of work, the petri dishes were then incubated for 48 hours in ambient temperature. All bacterial colonies (including those of pinpoint size) appearing on the agar medium were manually counted and recorded for each sample. Total bacterial counts were expressed as CFU/g calculated by multiplying the number of colonies on the plate by the dilution factor (1,000).

Determination of E. Coli Counts in the Samples

Five of the 36 samples were randomly selected for determination of counts of *E.* coli in the same manner as for *S. aurues*, using sterile petir dishes (plates) of MacConkey agar in place of Nutrient agar.

Statistical Analysis

The means of Aerobic Plate Count (APC) were first compared using the one-way ANOVA test. Assumptions of normality and equal variance were tested using the Shapiro-Wilk and Levene tests respectively. If the ANOVA demonstrated significance, more detailed results were found by comparing the means using unpaired t-tests. As two hypotheses were being tested on each data set, the Bonferroni-adjusted significance level of p=0.025 was used to correct for Type-I error. All analysis was made using actual data numbers; however, for simplicity, CFU results are reported by rounding to the nearest ten.

The ratios of "*E. coli* present" to "*E. coli* not present" were compared using Fisher's Exact Test as only five samples were used. Mean *E. coli* colony counts of each of the five samples were analyzed for normal distribution using Shapiro-Wilk test, and compared using unpaired t-test. A Bonferroni-adjusted p-value of .025 was employed.

Gram Staining

Loopfulls of round, white and yellow colonies appearing in the plates were suspended in sterile distilled water. Smears prepared by spreading a loopfull of the suspensions on microscopic slides were stained by Gram stain procedure using crystal violet as the primary dye and safranin as a counter stain following a standard procedure.

Results

Total Bacterial Counts

Total bacterial count of individual samples ranged from 1,200 to 282,000 CFU/g. Three of these from supermarket 3 fell outside the acceptable range of aerobic plate counts (below 30-300 CFU/g). The

Table 1: Total aerobic bacterial plate count in colony forming units (CFU/g) in 12 samples of ground beef from three supermarkets in Bonaire

Date of sample	Supermarket 1	Supermarket 2	Supermarket 3
11-Nov	193000	7200	10200
13-Nov	207000	5500	9200
15-Nov	233000	4200	9000
18-Nov	251000	3200	3900
20-Nov	276000	4100	2800*
22-Nov	205000	3900	4100
25-Nov	263000	3700	5600
27-Nov	220000	3900	1200*
29-Nov	259000	6400	4000
2-Dec	282000	5100	6200
4-Dec	38000	4800	5300
6-Dec	261000	5700	1900*

*CFU count per plate fell outside the generally accepted range of 30-300

counts in individual samples are shown in Table 1. Microscopic examination of Gram stained smears of different colored colonies showed the presence of both Gram positive and Gram negative bacteria in rods and sometimes in cocci. Since the study was aimed at determining the total bacterial counts in the samples, no record was kept of the relative umber of Gram positive and Gram negative bacteria. The mean APC CFU/g with 95% confidence interval are given in Table 2.

Table 2: Mean aerobic bacterial plate (CFU/g) with confidence intervals from 3 supermarkets in Bonaire

Supermarket	Mean CFU/g	95% Confidence Interval
1	224,000*	182,370-265,630
2	4,800	4,040-5,570
3	5,280	3,430-7,140

*Denotes that this mean is significantly higher than other means (p=1.488E-16)

Testing the One-Way ANOVA Assumptions

The Shapiro-Wilk Test

This test was employed to test the samples for normality. The bacterial count of 38,000 CFU/g from one of the samples from supermarket 1 (December 4) was found to be an outlier (defined as below Quartile 1-1.5xInterquartile Range). When the mean CFU/g for supermarket 1 was calculated without the outlier, the outlier was more than 6 standard deviations from the mean. The cause of this observation could not be known. It could represent an error in method or a pertinent observation, even if unlikely. For this reason, the data point was not dropped from the sample set completely. The analysis was run with and without this outlier. With the outlier included, supermarket 1 data yielded a Shapiro-Wilk statistic of W = 0.745 and a related p-value of 0 .0024 (n=12), indicating the population is not normally distributed. At the 5% significance level for normality, W = 0.859 is the critical value. With the outlier taken out of the data set, the 5% significance level the critical value is W = 0.850, supermarket 1 data yielded a Shapiro-Wilk statistic of W = 0.927 and a related p-value of 0.3823 (n=11), indicating normal distribution.

Supermarket 2 yielded a Shapiro-Wilk statistic of W = 0.941 and a related p-value of 0.5152 (n=12). At the 5% significance level, W = 0.859 is the critical value. Therefore, the null hypothesis was accepted that the population is normally distributed. Store 3 yielded a Shapiro-Wilk statistic of W = 0.937 and a related p-value of 0.4546 (n=12). At the 5% significance level, W = 0.859 is the critical value. Therefore, the null hypothesis was accepted that the population is normally distributed. As both supermarket 2 and supermarket 3 are normally distributed, we will accept the normal distribution of the data from supermarket 1 as well that was found excluding the outlier, keeping this exclusion in mind as we draw our conclusions.

Results from the Shapiro-Wilk test are represented in Table 3.

Table 3: Results from Shapiro-Wilk Test for Normal Distribution concerning APC CFU/g of raw ground beef samples from three supermarkets in Bonaire

Supermarket	W-Statistic	P-value	Critical value for W at 5% significance level	Result of Hypothesis
1*	0.927	0.3823	0.85	Accept, Normal Distribution
2	0.941	0.5152	0.859	Accept, Normal Distribution
3	0.937	0.4546	0.859	Accept, Normal Distribution

1* number of samples (n)=11. *Calculated without outlier. Store 2 and Store 3, n=12.

The final assumption for using one-way ANOVA analysis is that the variances are equal. The Levene Test for Equality of Variances yielded a W-statistic of 5.0029 (p=0.0129), indicating that the variances are different. However, the one-way ANOVA test is robust in this case as the sample sizes are equal; the effect on Type I error is minimal [11].With assumptions of independence, normal distribution and equal variances addressed, one-way ANOVA test was employed.

One-Way ANOVA

A one-way ANOVA test was run testing the

hypothesis that the mean APC CFU/g of all three samples were equal. The ANOVA yielded an F ratio of 133.717 and associated p-value of 1.488E-16. Therefore, the null hypothesis was rejected; at least one mean APC CFU/g was significantly different from the others.

Unpaired t-Tests

Mean APC CFU/g from supermarket 1 and supermarket 2 were compared. Using the unpaired t-test, the t-value was found to be11.588 (p=1.654E-07). This is well within the predetermined significance of p=0.025, indicating a significant difference between supermarket 1 and supermarket 2 means, with supermarket 1 having a much larger mean APC CFU/g for raw ground beef. An unpaired t-test comparing means from supermarket 1 and supermarket 3 yielded a t-statistic of 11.578 (p=1.653E-07),again indicating a significant difference between Store 1 and Store 3 means, with supermarket 1 having a much larger mean. The third unpaired t-test comparing means of supermarket 2 and supermarket 3 resulted in a t-statistic of -0.526 (p=0.609), indicating no significant difference between the means of supermarket 2 and supermarket 3. The results of the unpaired t-tests analyzing the means of the APC CFU/g are shown in Table 4.

Table 4: P-Values Derived from unpaired t-tests analyzing mean APC CFU/g of raw ground beef from three supermarlets in Bonaire n=12 for each supermarket

Null Hypothesis	t-Statistic	P-Value	Result
There is no difference between mean APC CFU/g of Store 1 and Store 2.	11.588	1.654E-07*	Reject Hypothesis; There is a difference.
There is no difference between mean APC CFU/g of Store 1 and Store 3.	11.578	1.653E-07*	Reject Hypothesis; There is a difference.
There is no difference between mean APC CFU/g of Store 2 and Store 3.	-0.526	0.609	Accept Hypothesis; There is no difference.

*Significance Level p =0 .025.

Occurrence of E. Coli in Ground Beef

Five samples from each of the three supermarkets were tested for presence of *E. coli*. E. *coli* was present in all five samples from supermarket 1, in four samples from supermarket 2, and in none of the five samples from supermarket 3. This resulted in a mean colony count of 52. *E. coli* was present in 3 of the 5 samples from supermarket 2, the colony counts being 26, 6, 0, 13 and 0, resulting in a mean colony count of 9. None of the five samples from supermarket 3 yielded *E. coli*.

Table 5: Observed mean *E. coli* colony counts from raw ground beef from three supermarkets in Bonaie

Sample no	Date of collection	SM # 1	SM # 2	SM # 3
1	8-Nov	96	26	0
2	27-Nov	30	6	0
3	29-Nov	69	0	0
4	2-Dec	37	13	0
5	4-Dec	28	0	0
Mean Colony Count		52	9	0

Table 6: Results from Shapiro-Wilk Test for Normal Distribution Concerning *E. coli* colony counts from raw ground beef samples. 5 from each of three supermarkets in Bonaire

From three supermarket	W-Statistic	P-value	Critical value for W at 5% significance level	Result of Hypothesis
#1	0.8496	0.1933	0.762	Accept, Normal Distribution
#2	0.8759	0.2909	0.762	Accept, Normal Distribution
#3	NA	NA	0.762	Included in analysis*

*For the scope of this study, we include mean=0 in analyzing data.

The data sets were tested for a normal distribution using the Shapiro-Wilk test. Store 1 yielded a statistic of W=0.8496 (p=0.1933), indicating normal distribution. Store 2 yielded a Shapiro-Wilk statistic

of W = 0.8759 (p=0.2909), also indicating normal distribution. Store 3 presented an interesting situation. A total of zero counts were observed with no standard deviation or variance. Therefore, a Shapiro-Wilk statistic could not be found, as division by zero is undefined. For the limited scope of this study, we included the data from marker 3 anyway and kept this in mind in concluding our study. No outliers were found in the data sets.

Results from Shapiro-Wilk test are represented in Table 6.

With the assumption of normal distribution met, unpaired t-tests were employed on the data. comparing means of supermarket 1 and supermarket 2, a t-statistic of 3.229 (p=0.032). This is above the Bonferroni-adjusted significance of p=0.025. Therefore, we must accept the hypothesis as it relates to our sample. There is not a significant difference represented between the mean colony counts of supermarkets 1 and 2. Mean *E. coli* colony counts of Store 1 and Store 3 were compared yielding a t-statistic of 3.638 (p=0.022), indicating a significant difference. The test comparing markes 2 and 3 indicated no significant difference between means with a t-statistic of 1.844 (p=0.139).

The results of the unpaired t-tests analyzing the means of *E. coli* colonies are represented in Table 7.

Table 7: P-Values Derived from Unpaired t-tests analyzing mean *E. coli* colony counts (ECC) of raw ground beef

Null Hypothesis	t-Statistic	P-Value	Result
There is no difference between mean ECC of Store 1 and Store 2.	3.229	0.032	Accept Hypothesis; There is no difference.
There is no difference between mean ECC of Store 1 and Store 3.	3.638	0.022	Reject Hypothesis; There is a difference.
There is no difference between mean ECC of Store 2 and Store 3.	1.844	0.139	Accept Hypothesis; There is no difference.

No of samples for each supermarket, Bonferroni-adjusted significance level p=0.025.

E. coli was Found in All the 5 Samples From Supermarket 1, and in 2 Samples Each from Markets 2 and 3.

The proportions of presence of *E.coli* were compared using Fisher's Exact Test. The data was independent, there are no outliers and the variables meet the classifications for use in the contingency table of Fisher's Exact Test. The test comparing proportions of *E. coli* colony present in samples from market 1 and 2 yielded a p-value of 0.4444, which is above the Bonferroni-adjusted significance of p=0.025, indicating no significant difference between proportions from Store 1 and Store 2. The test comparing proportions from markets1 and 3 yielded a p-value of 0.0079, indicating a significant difference between proportions. Comparison of proportions from markets 2 and 3 yielded a p-value of 0.1667, indicating no significant difference between the proportions.

These hypotheses, p-values and results concerning proportions of *E. coli* presence as found using Fisher's Exact Test are represented in Table 8.

Table 8: P-values derived from Fisher's Exact Test analyzing proportions of presence of *E. coli* colonies in samples of raw ground beef

Null Hypothesis	P-Value	Result
There is no difference between the proportions of *E. coli* presence in beef from Store 1 and Store 2.	0.4444	Accept Hypothesis; There is no difference.
There is no difference between the proportions of *E. coli* presence in beef from Store 1 and Store 3.	0.0079	Reject Hypothesis; There is a difference.
There is no difference between the proportions of *E. coli* presence in beef from Store 2 and Store 3.	0.1667	Accept Hypothesis; There is no difference.

No. of samples for each supermarket=5, Bonferroni-adjusted significance level p=0.025. Note: Fisher's Exact Test does not have a "test-statistic" but computes the p-value directly.

Results Summary

It was found that the means of APC CFU/g of the sampled raw ground beef were different. Specifically, the raw ground beef from Store 1 has a mean APC CFU/g that is higher than both Store 2 and Store 3 at a p=0.025 significance level, while the means of APC CFU/g from Store 2 and Store 3 are not statistically different from each other. Additionally, the only difference in proportions of *E. coli* presence and mean colony counts at a significance level of p=0.025 was found between Store 1 and Store 3, with Store 1 having a higher proportion of presence and mean colony

count of *E. coli*. The presence of Gram-negative bacteria was seen in each of the six Gram stained slides.

Discussion

Studies of this nature that assess the quality of ground beef using total bacterial counts and *E. coli*as markers have been done in several parts of the world [3,4,5,6,12,13]. It is interesting to note that our results pertaining to total bacterial countl fall within standards of health as suggested by the Institute of Food Science and Technology (IFST) [14]. The IFST suggests that the maximum level for CFU/g obtained from APC at any point in the shelf life of a raw meat product is 10^7 colonies per gram of meat. This is good news for the consumers and producers of raw ground beef in Bonaire. However, there is still a significant difference between the beef from Store 1 and the other two locations. Additionally, the means of APC CFU/g from Store 2 and Store 3 are similar to numbers reported in nationwide US studies of raw ground beef, while the mean APC CFU/g from Store 1 is considerably larger [5,6]. For example, the mean APC CFU/g from our Store 1 would have fallen within the top 6.3% of 1,719 samples reported in one US study [5]. This suggests that although the Store 1 data is within limits due to health, its overall bacterial levels are greater than normal. As discussed earlier, this can negatively impact the shelf life of the meat [7] and poses possible health concerns for Store 1 consumers who undercook their beef or allow cross contamination.

Future research could be more quantitative and specifically address the relatively poor bacteriological quality of the beef retailed in supermarket 1 (total bacterial count/g and E. oli count/g being higher than in that from the markets 2 and 3) Questions could be answered such as: Addressing the questions:What are the handling procedures of raw ground beef in Store 1 versus the other stores? What is the timeframe of beef processing and sales? These kinds of questions could help Store 1 address the specific differences in procedure that may be leading to increased total bacteria and *E. coli* in their ground beef, thus allowing Store 1 to improve their product.

Limitationss

We predict that most objections to this study will be because of the relatively smaller sample sizes and narrow scope, which were part of the limitations of this particular study. While *E. coli* was detected in several of our samples, there arr various different kinds of *E. coli*, many of which are opportunistic [15]. Therefore, we cannot make direct conclusions as to the health risk involved, only that further research needs to be done. The presence of Gram-negative bacteria in the Grram-stained slides, along with the presence of *E. coli* in the raw beef samples, suggest that other gram negative pathogenic bacteria such as *Salmonella* or *Shigella* may be present in the beef. While it was not within the scope of this study to further describe specific bacteria, this is an excellent suggestion for further research. Future studies could focus on other bacteria often present in ground beef such as *Salmonella*, *Shigella*, *Campylobacter jujuni*, *Listeria*, *monocytogenes* or *Staphylococcus aureus* which along with *E. coli* can all cause disease or death [1,2]. Research could select for specific types of *E. coli*, including 0157:H7 as this strain is particularly connected with human illness and is usually meant to be undetectable in raw meat [3,15,16]. Gram-negative bacteria as a general group are more resistant to antibiotics than gram-positive bacteria [17]. Research could also address whether or not all of these bacteria present in the beef are resistant to antibiotics, a growing concern [3].

Another objection to our study could be the handling of the outlier observed in the Store 1 data. Further research, increasing the samples of total bacterial count, could bear on our decision to assume normality of distribution. The same principle applies to the low counts of TBC observed in three of the Store 3 samples; our study results will become more significant (or less) as future research increases the studies of total bacterial counts.

It was only within this study scope, and therefore, a limitation, to include three of the major supermarkets on the island of Bonaire with a relatively short time-frame of sample collection. Samples were taken over one month's time and not throughout a whole year. For this reason, our analysis may not reflect possible seasonal differences [6]. Clearly, there are also other sources from which consumers obtain their meat, beyond the three selected supermarkets. These other stores could undergo these studies as well and over a longer time-frame to form an even more complete picture of the quality of the raw ground beef for sale in Bonaire.

Conclusion

Consumers have many options when it comes to purchasing meat and it will be an advantage for them

to have access to the data from this study which compares levels of bacterial counts in locally available meats. From this study, local consumers can gain a better understanding of which store provides the highest quality and safety relative to total bacterial count and then weigh other factors such as convenience and cost and make consumption choices that best meet the needs of their circumstances.

This study also provides a framework for the supermarkets to assess their own requirements for purchase and handling of meat. Stores with higher quality as related to total bacterial count can use that as a selling point for their consumers, can find pride in providing quality products and can continue to use safe meat handling procedures. Stores with lower quality meat can be made aware of the findings and assess the practices leading to these circumstances. This information may be used to implement improved meat handling guidelines and thereby benefit the supermarket and consumer alike.

Finally, studying the quality of local raw ground beef through *E. coli* and aerobic plate counts can be a platform for encouraging safe handling of ground beef in Bonaire. As people come to understand that raw ground beef does contain bacteria, they may be motivated to follow safe meat handling guidelines. These guidelines include keeping meat cold and clean until cooking, washing surfaces before and after coming into contact with the raw meat, and cooking meat to safer temperatures [7]. This research can serve as a foundation for education and more studies dealing with meat safety and food poisoning prevention.

This study aimed to answer the question, "Is there a difference between the total bacterial counts of raw ground beef sold at three of the major supermarkets in Kralendijk, Bonaire, Dutch Caribbean?" Our null hypothesis was that there was no difference in the total bacterial counts in meats available to consumers. To a lesser degree, this study also assessed whether *E. coli* bacteria were present in the meat sold at the same three supermarkets. The null hypothesis was that no *E. coli* was present and that there was no difference between the three supermarkets.

Consumers and the supermarkets can benefit from these findings in many ways.

This study serves as a good foundation in beginning to assess the overall quality of raw ground beef in Bonaire. Statistical comparisons allow us to see that there are differences in the means of total bacterialCFU/g between different supermarkets. This information allows consumers to recognize where the highest quality of beef can be obtained as it relates to contamination, and to a lesser degree, overall prevalence of *E. coli*. The study can help specific producers see that improvementscan be made in their product. In general, the overall presence of bacteria and specifically*E. coli* should encourage the safe-handling of meat by everyone involved in the process. Areas for further research are suggested as a means of building upon the groundwork laid here.

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A Study of Community-Acquired Methicillin-Resistant Staphylococcus Aureus Infections among College Students

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Abstract

Introduction: Staphylococcus Aureus (*S. aureus*) is a leading cause of gram positive bacterial infections and produces a wide spectrum of diseases. Although *S. aureus* infections were historically treatable with common antibiotics, emergence of Methicillin-resistant *Staphylococcus Aureus* (MRSA) in 1960 in hospital-associated settings, and later in community settings imposed a great burden on health care resources. Several reports have documented MRSA outbreaks in hostels, dormitories and military barracks among inmates. Present study was undertaken in April-May 2015 to investigate an outbreak of CA-MRSA among hostel inmates in Mangalore. *Material and Methods:* Present study was carried in a nursing college hostel in Mangalore, after its first student was admitted on 04 Aril 2015 with severe skin infection caused by MRSA. Subsequently, 291 suspected hostel inmates were examined and their nasal and skin swabs were sent to hospital laboratory for culture and sensitivity. *Results:* Out of 291 swabs sent for culture, 51(17.52%) were found to be MRSA positive. Out of these, 34 (66.66%) were nasal and 17 (33.34%) were skin swabs. All isolates were found to be resistant to penicillin, ampicillin and erythromycin, but susceptible to ciprofloxacin, clindamycin, doxycycline, tetracycline, co-trimoxazole and vancomycin. These had the drug-resistance pattern of CA-MRSA strains. *Conclusion:* Community-based surveillance studies are required to understand how MRSA is transmitted in the community. Besides, development of an epidemiologic surveillance system would further identify CA-MRSA prevalence and the associated risk factors.

Keywords: CA-MRSA; Culture; Resistance; Outbreak; Prevention.

Introduction

The world is headed for a post-antibiotic era, in which common infections and minor injuries which have been treatable for decades can once again kill," says Dr Keiji Fukuda, WHO's Assistant Director-General for Health Security. Unless significant actions are taken to prevent the misuse of antibiotics, the implications may be devastating [1]. *Staphylococcus Aureus* (*S. aureus*) is a leading example. Although *S. aureus* infections were historically treatable with common antibiotics, emergence of Methicillin-resistant *Staphylococcus Aureus* (MRSA) in 1960 in hospital-associated settings, and later in community settings imposed a great burden on health care resources [2]. It has been observed that people with MRSA (methicillin-resistant *Staphylococcus aureus*) infection are 64% more likely to die than people with a non-resistant form of the infection [3]. Jeanine Thomas a survivor of MRSA sepsis in USA, was instrumental in passing of resolution in 2009 officially designating 02 October as "World MRSA Day" and October as "World MRSA Awareness Month" [4].

Though MRSA began as a hospital-acquired infection (HA-MRSA) ,it later developed limited access to the community (CA-MRSA) as well . These infections often occur at sites of cuts or scrapes in the skin, as well as in areas of the body covered with hair. Some CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional HA-MRSA infections, and they may affect vital organs and lead to widespread infection (sepsis), toxic shock , necrotizing pneumonia and even death.[5] Even though CA-MRSA may affect anyone , but it is more common among athletes, prisoners, and other groups of people who live in

crowded settings and/or routinely share contaminated items. Poor hygiene practices, such as lack of hand washing, may spread the bacteria more easily [6]. Outbreaks have generally been seen among military recruits, day-care attendees, injection-drug users and gay men [7].

There has been an outbreak of CA-MRSA in Nursing College hostel inmates of AJIMS & RC, Mangalore in April- May 2015. The outbreak was investigated and measures to control it were promptly instituted.

Material and Methods

Present study was carried in nursing college of Laxmi Memorial Education Trust, Mangalore, after its first student was admitted on 04 Aril 2015 with severe skin infection caused by MRSA. Subsequently, 291 suspected hostel inmates were examined and their nasal and skin swabs were collected and sent to hospital laboratory.

Two samples were collected from each student from two body sites i.e. nose and hand. For nasal sample collection, a sterile cotton swab was moistened by inserting into sterile saline solution and then inserted into both anterior nares, one at a time with the same swab, and rotated gently against the inner surface. Skin samples from hand were collected with a moist swab prepared as described for nasal sampling, by gently rubbing the fingertips of one hand . Nasal and skin samples were collected from 291 students .Antibiotics tested included penicillin, ciprofloxacin, clindamycin, erythromycin, doxycycline, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.

Results

The index case, a nursing student was admitted on 09 Aril 2015 in the hospital with severe skin infection caused by MRSA while the last case was reported on 24th May 2016 (Figure 1 A & B).

Out of 291 swabs sent for culture, 51(17.52%) were found to be MRSA positive. Out of these, 34 (66.66%) were nasal and 17 (33.34%) were skin swabs. All isolates were found to be resistant to penicillin, ampicillin and erythromycin, but susceptible to ciprofloxacin, clindamycin, doxycycline, tetracycline, co-trimoxazole and vancomycin. These had the drug-resistance pattern of CA-MRSA strains (Table 1).

The main lesions reported by the patients were Furuncle (33.00 %) ,Impetigo (29.62%),abscess (22.22%) and cellulitis (14.81%) (Figure 2).

Hospital records of preceding three years i.e. 2012 – 2014 were also analysed and it was observed that highest number of cases were recorded in the month of July i.e. 07; while maximum number of cases were reported during 2012 (33),followed by 2014 (21) while the lowest number of cases were admitted during 2013(19) (Figure 3).

All students found positive for MRSA were given appropriate treatment. Swabs were repeated 48 hours after the completion of treatment and thereafter at weekly intervals for three consecutive weeks. All other hostel inmates were de-colonized. Hand hygiene practices by the staff and students were stepped up. Hand rubs were provided in portable sizes. The Staff was sensitized about the outbreak and its nature.

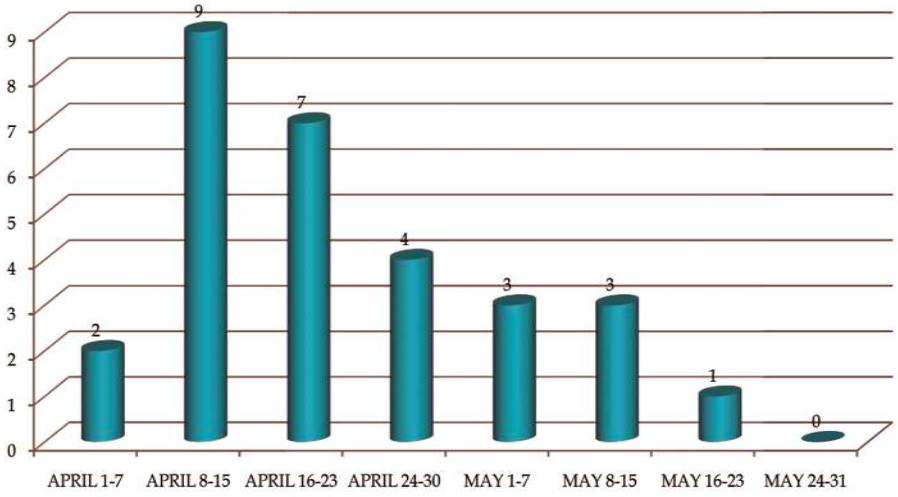

Fig. 1a: Distribution of cases during the month of April-may: 2015

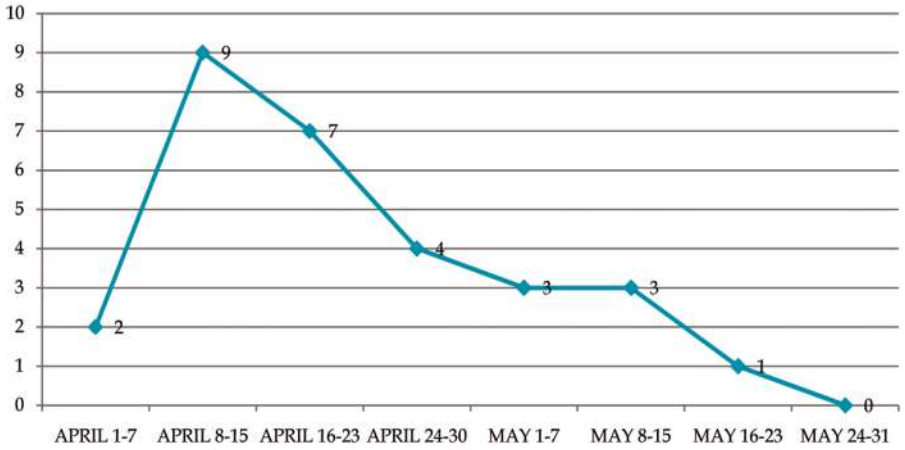

Fig. 1b: Distribution of cases during the month of April-may: 2015

Table 1:

MRSA: CULTURE AND ANTIBIOTIC SENSITIVITY								
PE	CI	CL	DO	TE	TS	AMP	VA	ER
R	S	S	S	S	S	R	S	R

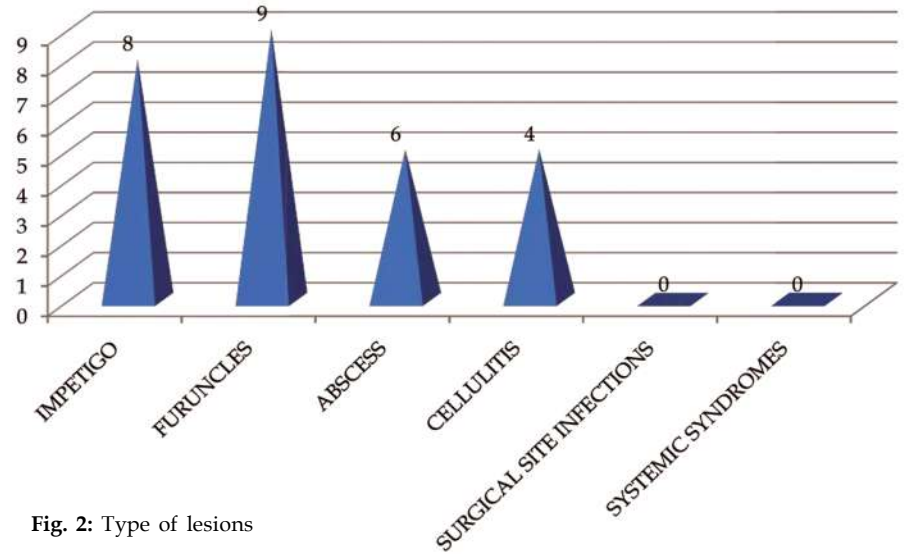

Fig. 2: Type of lesions

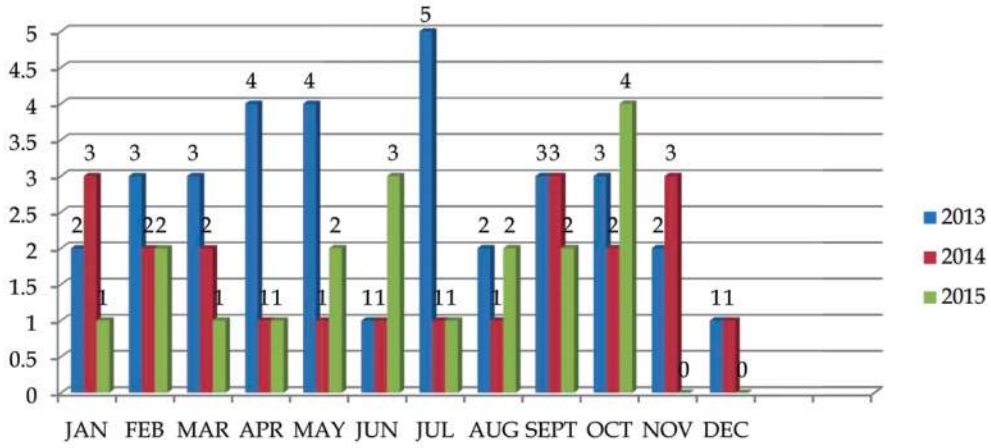

Fig. 3: Hospital records (2012 – 2014)

Discussion

Community-associated MRSA infections (CA-MRSA) are MRSA infections in healthy people who have not been hospitalized or had a medical procedure (such as dialysis or surgery) within the past year. First recognized in 1960, these were considered to be a medical oddity [8]. Now, MRSA is the most common nosocomial bacterial pathogen isolated in many parts of the World. In the past, community-acquired MRSA (CA-MRSA) infections tended to occur in patients with frequent health care contact or, less commonly, in specific groups of patients, such as intravenous drug users [9]. During the past decade, however, there has been a dramatic change in the epidemiology of community-onset infections caused by MRSA [10]. Young, healthy individuals who lack classic risk factors for MRSA infection are often affected. In present study the prevalence of CA-MRSA was found to be 17.52 % with nose being the more common site(66.66%) than skin (33.34%) (Figure 1). S. K. Fridkin et al (2005) [11] in their study among 1647 cases of CA- MRSA infection observed a prevalence between 8 to 20 percent .Similar findings were also reported by T. J. Ochoa, et al and S. Bratu et al and other researchers in their studies [12-16]. However Joshi S et al (2013) [17] in their study among 26310 isolates found an overall prevalence of 41% while majority of *S. aureus* isolates was obtained from patients with skin and soft tissue infections .Similar results have also been reported in other studies [18,19].

According to a WHO report on antimicrobial resistance high rates of resistance in common infections (e.g. urinary tract infection, surgical site infections, pneumonia and bloodstream infections) have been reported all over the world particularly healthcare associated infections while in MRSA, it is as high as 44% in some parts of the world [20].

In present study all isolates were found to be resistant to all β-lactam antibiotics (beta-lactam antibiotics) and cephalosporins. However, these were found to be sensitive to clindamycin, trimetho-sulfa, Vancomycin, Ofloxaciline, doxycycline, minocycline, rifampin and linezolid . According to the U.S. Centers for Disease Control (CDC), in 2004, 63% of all reported staph infections in the United States were caused by MRSA [21]. The figure represents a remarkable 300% increase in just 10 years' time. (In 1995, about 22% of all reported staph infections were MRSA, compared with only 2% in 1974). Needless to say, physicians no longer prescribe traditional antibiotics for methicillin-resistant staph infections (Micet, 2007). Instead, they usually administer "last-resort" intravenous vancomycin, although a growing number of doctors are now prescribing other newer antibiotics. Even with these options, scientists estimate that about 19,000 people in the United States die every year from MRSA. This figure is more than the number of U.S. residents and citizens that die from HIV/AIDS (about 17,000 every year) [21]. In another study by Dr. Pablo et al found less than 20% of S. aureus which were isolated mostly from the wounds and abscesses of admitted patients, exhibited resistance to chloramphenicol, tetracycline, and ciprofloxacin but with excellent susceptibility to linezolid [22]. The susceptibility test also confirms a high and increasing prevalence of MRSA and ICR among admitted patients [24].

CA-MRSA strains can produce a variety of lesions i.e. from impetigo to life-threatening necrotizing fasciitis .However, abscesses and cellulitis are the most common presentations i.e. 50%–75% of patients present with abscesses, while 25%–50% with cellulitis generally as single lesions over the extremities [10]. Folliculitis caused by CA-MRSA is a less frequent form of presentation usually with erythe-matous folliculocentric pustules, which can compromise uncommon localizations (e.g., periumbilical). Impetigo and scalded-skin syndrome due to CA-MRSA (usually in children) are also uncommon forms of the disease. Pyomyositis and myositis due to CA-MRSA are uncommon infections usually involving the lower extremities or pelvis [25]. The main lesions reported by the students in present study were Furuncle (33.00 %) ,Impetigo (29.62%), abscess (22.22%) and cellulitis (14.81%).

Hospital records of preceding three years i.e. 2012 – 2014 were also analysed and it was observed that highest number of cases were recorded in the month of July i.e. 07; while maximum number of cases were reported during 2012 (33), followed by 2014 (21) while the lowest number of cases were admitted during 2013(19) (Figure 3).

All students found positive for MRSA were given appropriate treatment .Their swabs were repeated 48 hours after the completion of treatment and thereafter at weekly intervals for three consecutive weeks. All other hostel inmates were de-colonised. Hand hygiene practices by the staff and students were stepped up. Hand rubs were provided in portable sizes. The Staff was sensitized about the outbreak and its nature

Conclusion

MRSA is a global health problem causing

infections in hospitals as well as in the community. It also remains one of the most important causes of Health Care Associated infections worldwide. Moreover, many MRSA strains have developed resistance to most of the available antibiotics. MRSA carriers also serve as reservoirs for further transmission as they move through and across healthcare facilities. Needless to say that, with strict adherence to basic infection-control practices i.e. hand hygiene, observance of universal precautions, early identification of cases, isolation and de-colonisation of infected patients; it is possible to bring down the MRSA transmission to minimum levels. Besides, the knowledge of the risk factors, transmission mechanism, preventive measures and local epidemiology of MRSA, will further help in improving compliance.

Limitation

The study had the limitation of not undertaking the sub typing of MRSA strains needed to identify specific strains of MRSA as many studies have found that CA-MRSA strains in India carry the Panton-Valentine leukocidin (PVL) virulence factor and has staphylococcal cassette chromosome mec (SCCmec) type IV and SCCmec type V .

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Detection of S*taphylococcus Aureus* for Food Safety in Ground Beef in Supermarkets in Bonaire (Dutch Caribbean)

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Abstract

Background: There is lack of information on staphylococcal contamination of foods in the Dutch Caribbean. The aim of this study was to evaluate retailed ground beef for contamination with *Staphylococcus aureus* in Bonaire (Dutch Caribbean). *Methods:* Fifty-one samples of ground beef collected from three different supermarkets in Bonaire were inoculated on Mannitol Salt Agar (MSA) for selective isolation of S*taphylococcus aureus*. Aseptic techniques were used for collection and processing of samples. *Results:* The isolates tentatively identified as *S. aureus* on MSA were confirmed by characteristic microscopic morphology in Gram stain and by production of catalase and coagulase. The presence of *Staphylococcus aureus* in ground beef samples from the three markets varied in quantities from 130 to 300 colony forming units/g. *Conclusion:* The detection of *S. aureus* in high quantities in samples of retail ground beef emphasizes the public health importance of this work. The public health authorities in Bonaire should alert the management of the supermarkets of this finding, and urge them to take precautionary measures to avoid or minimize bacterial contamination.

Keywords: Ground Beef; Bonaire; *Staphylococcus aureus* Count

Introduction

Staphylococcus aureus is a major medically important species of Gram positive cocci. Staphylococcal diseases range from local infections manifesting as abscesses, carbuncles, boils to systemic infections like pneumonia, subacute endocarditis, osteomyelitis, and food poisoning [1, 2]. *S. aureus* occurs as a component of the normal microflora of nose, pharynx and sometimes skin [2]. This organism produces a variety of toxins, viz.(SEs; SEA to SEE, SEG to SEI, SER to SET with demonstrated emetic activity [3]. When these bacteria contaminate food and are allowed to grow, they secrete enterotoxin, ingestion of which can cause food poisoning. The incubation period of staphylococcal food poisoning is short i.e. 4-6 hours because the enterotoxin in the food has already been formed by the staphylococci before the food is ingested. Staphylococcal enterotoxins are super-antigens and cause gastro-intestinal symptoms such as nausea, vomiting and diarrhea. Illness is acute and usually mild, and can regress after one to three days by itself [3]. The aim of this study was to detect the presence of *Staphylococcus aureus* in samples of ground beef sold in three big supermarkets in Bonaire

Methodology

Fifty-one samples of ground beef from three different supermarkets located in Bonaire, Dutch Caribbeanwere collected aseptically in ziplock polythene bags surface sterilized from inside and outside by swabbing with 70% isopropyl alcohol. The markets were labelled as #1, #2, and #3 for privacy concerns. The samples from these markets were examined over a period of several weeks in the month of November – December, 2013 for presence of staphylococci. Altogether 17 samples from each of the supermarkets were investigated. The samples were handled using sterile gloves, hand sanitizer, and 70% isopropyl alcohol was used in cleaning surface area of the working bench before and after processing of the samples to prevent bacterial contamination.The shelf life of the samples was not provided by the operators of the super markets. The

samples were processed immediately after collection. The inoculating loops were sterilized with the use of Bunsen burner prior to inoculation. Two mm loopful's of each sample (equivalent to one gram) were inoculated on three plates of Mannitol Salt agar (MSA) (allowed to warm to room temperature and the agar surface to dry before inoculating) by streaking according to standard streaking procedure, for selective isolation of S*taphylococcus aureus*. MSA was used as a selective medium for the study as this medium is recommended by the American Public Health Association for the enumeration of staphylococci in food and dairy products[4]. The inoculated plates were incubated for 24-36 hours mostly at 37^0 C in The Bon Lab, Krandejik. At times when the incubator in BonLab was not accessible, the plates were incubated at room temperature ($27\text{-}32^0$ C). Incubating at the latter temperatures did not affect the growth of Staphylococci. The number of colonies suggestive of *S. aureus* in the inoculated plates of MSA was counted manually or sometimes with the help of colony counter available in Bon Lab. The average of counts on three plates inoculated with each sample was computed.

Results

The isolates tentatively identified as S. aureus on MSA on the basis of yellow colouration of the colonies were further studied by Gram-stain and tested for catalase, and coagulase. All isolates showed microscopic morphology as Gram positive cocci in irregular clusters and were positive for production of catalase and coagulase, thus confirming their identity as *S. aureus*. The presence of *Staphylococcus aureus* in ground beef samples from the three markets varied in quantity from 130 to 300 colony forming units (CFU)/g, as given in the table below.The differences in counts of CFU in the samples from the three markets were statistically significant, as a comparison of the data by ANOVA showed a p-value of 6.92682. *S. aureus* was most frequently recovered from market #1, followed by markets #2 and #3.

Table 1: No. of colony forming units (CFU) per gram in 17 consecutive samples of ground beef from three supermarkets in Bonaire

Serial no. of sample	Average number of CFU		
	Supermarket #1	Supermarket #2	Supermarket #3
1.	230	170	130
2.	130	230	200
3.	270	230	170
4.	170	200	100
5.	230	200	170
6.	270	270	170
7.	270	170	170
8.	300	170	130
9.	270	170	200
10.	270	170	170
11.	300	300	170
12.	270	170	130
13.	270	170	230
14.	300	170	230
15.	230	130	170
16.	230	170	200
17	230	200	130

Discussion

S. aureus food poisoning is one of the commonest causes of food borne illnesses world-wide, resulting from preformed enterotoxins in foods contaminated with the organism, and is one of most frequent causes of reported cases of food poisoning in USA [5]. Investigation of the sources and implications of *S. aureus* in retail ground beef is important. The public should be educated on safe beef-handling practices. It is known that not all strains of *S. aureus* are toxigenic [3]. Though we did not test our isolates of *S. aureus* for toxin production, it is possible that some of them were toxin producers. Also methicillin resistant S. aureus may be present in retail ground beef as reported from Georgia, USA [6]. This together with the fact that the contamination of the retail beef with *S. aureus* was very high in terms of CFU/gm emphasizes the public health importance of this work. Some investigators have found major source

of contamination of ground beef with *S. aureus* to be the hands of workers who slaughter the cattle [7]. The results of this study can later be presented to various health organizations and professionals on the island if needed to motivate them to enhance their food bacterial monitoring system to prevent outbreaks of food-borne illnesses.

Conclusion

The presence of *S. aureus* in high quantities in samples of retail ground beef in supermarkets in Bonaire is of public health concern. The public health officials in Bonaire should draw the attention of the management of the supermarkets to this finding for enforcing hygienic precautions to avoid bacterial contamination of meat products.

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Awareness of AIDS amongst High School Students in Bonaire, Dutch Caribbean

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Abstract

Background: The Caribbean region is the second most affected region in the world in terms of rates of HIV prevalence after sub-Saharan Africa. HIV/AIDS education needs to target at the young age groups. The aim of the study was to investigate the awareness of AIDS amongst high school students in Bonaire. We hypothesized that after a thorough workshop for students (teens) in schools, they will have a better understanding of the transmission of AIDS and its prevention. *Methods:* Fifty-two students were tested by using Healthy Oakland Teens Survey, two times, initially and after conduction of workshop on AIDS for the students, in order to see efficiency of this education effort to improve knowledge of AIDS. *Results:* Knowledge of the students about AIDS significantly increased after conduction of the workshop as compared to that before the workshop. There was a decrease in the number of students stating they were uncomfortable having a conversation about the sex, AIDS and its protection with friends, parents and adults from 32% before the workshop to 26% after the workshop. Their openness for conversation about AIDS, its protection, and sex with friends, adults, and others in the social community also significantly increased after workshop as compared to that before the workshop. *Conclusion:* There is need for creating enhanced knowledge and awareness on HIV/AIDS among adolescents. It is crucial not only for protecting the students from AIDS but also for preventing the spread of HIV infection.

Keywords: AIDS; Awareness; High School Students; Bonaire.

Introduction

Acquired Immune Deficiency Syndrome or better known as AIDS is still a worldwide concern and a cure has yet to be found. AIDS has become pandemic and now is being spread throughout the world [1]. In 2014, there were 36.9 million [34.3 million–41.4 million] people living with HIV in the world; this included 280 000 [210 000–340 000] people with HIV in the Caribbean. The first Caribbean case of AIDS occurred in Jamaica in 1982 [2]. In the early days of the epidemic, more men were affected than women [2]. The Caribbean is the second most affected region in the world in terms of HIV prevalence rates after sub-Saharan Africa, the leading cause being heterosexual sex [3]. Besides Africa, the Caribbean is the only place in the world where a higher number of girls and women (than boys and men) have HIV infection [3]. Several factors influence this epidemic, including poverty, sex tourism, social vulnerability arising from poverty, illiteracy or limited education, unemployment, and gender inequality. A study conducted by CDC's Youth Risk Behavioral Survey (YRBS), revealed that many young people start having sexual intercourse at early ages; 47% of high school students have had sexual intercourses before the age of thirteen [4]. Previous research has revealed that a large percentage of adolescents are not worried about becoming infected with AIDS/HIV [4]. A study done from 2001–2004 concluded that 62% of the 17,824 persons 13–24 years of age diagnosed to have HIV/AIDS were males, and 38% were females [5]. Gender plays an important role in the spread of HIV. Young women are more likely than men to contract HIV in the Caribbean, and most of these women are

between 24–44 years old [5], Young girls in the Caribbean are at particularly high risk of becoming infected at very young age, especially by older men who are much more likely to be HIV infected than their younger counterparts. One of the factors that put women most at risk is sexual violence. In general, violence against women as well as sexual abuse of young men and children are increasing in the region. Surveys indicate that some 21% of boys and 18% of girls may have been sexually abused before they are 16 years old, and 1% of men and 6% of women are sexually abused as young adults [6].

According to Pan American Health Organization report [7] between 1985 and 2010, 2,147 persons in the former Netherlands Antilles (including Aruba, Bonaire and Curacao) tested positive for HIV, of whom 57.3% were males and 42.7%, females. Most cases were concentrated in Curacao (1,426 cases, 66.4%) and Sint Maarten (664 cases, 30.9%). Approximately 63% of HIV transmission in men was through heterosexual contact. The demographic, ethnographic and geo-climatic features of Bonaire are similar to that of other Dutch Caribbean islands with a high tourist economy as well as a high prevalence of HIV/AIDS. There are several factors that should alert on importance of HIV in Bonaire, viz. a great number of tourist visitors from Europe, North America, South America, and neighboring islands. Numerous people from Dominican Republic work in Bonaire as cleaners, bartenders, waitresses and tourist guides. These workers often offer sexual favors to tourists they meet in their work place.

There is a high level of sexual activity among the youth, as evidenced by the 22 to 32 percent of persons in six eastern Caribbean states reporting having sex before 13 years of age [8]. It is thus imperative for parents, teachers, and all concerned to educate the young generation in protecting the society from AIDS [9]. We hypothesize that after a thorough workshop with teenage students, they will have a better understanding of the transmission of AIDS and escalation and spread of this infection. We believe schools can play a leading role for reaching youth before high-risk behaviors are established.

Methods

The study included 52 participants, 29 males (55.77%) and 23 females (44.23%) from the high school. We used Healthy Oakland Teens Survey [10]. The questionnaire was done two times, initially and after conduction of workshop on AIDS for the students, in order to see efficiency of this education effort to improve knowledge of AIDS. The entire questionnaire was explained to the student participants and all queries raised by them were clarified. The first part of survey provided demographic data: sex, age, race, with whom they live, primary language and comfort ability with English, and their sexual behavior. The second part included questions to test the knowledge of students about AIDS in terms of saying "True", "False", and "Don't know". In third part, the students were asked to state if they felt comfortable to have a conversation about AIDS, protection, and sex with friends, and others. The fourth included basic questions to determine level of understanding on how efficient are condoms to prevent HIV infection. In fifth set questions related to students and their friends' attitude to sexuality. Sixth set dealt with students' attitude to discuss with parents about sexuality. Seventh part was about their concern to get infected with AIDS, and finally eighth part concerned their personal opinions about wearing condoms.

This study was conducted with the permission of Bonaire Department of Health, and with clearance from the ethical and review committee of Saint James School of Medicine, Bonaire. Informed consent was obtained from each of the students.

Results

Responses to the questions about the general knowledge of the students before and after the workshop are given in Table 1, while responses to questions related to condoms before and after workshop are given in Table 2. The responses are also depicted in Figures 1-4. The results of the study are mentioned below under different subheads.

Demographic Data

The students were aged 13-18 yrs with mean age as 15.5 yrs, 55% were males and 45% females. 86% of the students were Bonairian, the rest from other nationalities. 71% had Papiamentu as their primary language, 29% had English, Spanish or Dutch as their primary language. 55% of the students lived with both parents, 32% lived with mother only, while 8% lived with father only, and 5% lived with another relative.

Behavioral Data on Sexuality

92% students had been out on dates. 78% had French kissed the dated partner; 40% have had sexual

intercourse with average age of 15 years at first intercourse, and 28% participated in sexual activity at least once a month. Only 21% used condoms during sexual act. 90% of the students have had alcohol at least once; average age of first drink being14 yrs. 74% of the students had not yet had a conversation with their parents about sex and AIDS. 37% stated "other" as the source for knowledge of AIDS; however, they did not specify what other source was and 32% stated they heard about AIDS from siblings or teenage relatives. The data on the responses of the students on the questions 3a to 3k are depicted in Table 2.

Responses to the first set of questions on general statements about AIDS. (Students were asked to choose the option True, False, or Don't Know)

Before the workshop, on average 34% stated "Don't Know" as the answer to the first set of questions. On average 38% of the students answered at least one of the questions correctly. After the workshop, on average 8.7% stated the answer as "Don't Know". Further on average 66% of the students answered at least one question correctly. Thus there was a significant increase compared to the results prior to the workshop

In order to test if there was statistically significant difference before and after workshop, Nonparametric Wilcoxon Signed Ranks Test in statistical package SPSS was used. Statistically significant difference before and after workshops showing increase of knowledge about AIDS was found for following questions:

Question 3a: Only people who look sick can spread the AIDS virus. ($p = 0.018$; $p < 0.05$).

Question 3b: Condoms reduce the risk of getting the AIDS virus. (A condom is a piece of rubber that fits over the penis.). The higher rate of correct response after the workshop was almost statistically significant ($p = 0.054$). This means that even greater stress should be put on importance of prevention by using condoms.

Question 3e: Most people who have the AIDS virus show signs of being sick right away. ($p = 0.046$; $p < 0.05$).

Question 3f: You can get AIDS by having anal sex without a condom (By anal sex we mean putting a penis in another person's anus [butt]. ($p = 0.003$; $p < 0.01$).

Question 3g: You can get AIDS by being bitten by a mosquito that has bitten someone with AIDS. ($p = 0.007$; $p < 0.01$).

Question 3h: Only people who have sexual intercourse with gay (homosexual) people get AIDS. ($p = 0.001$; $p < 0.01$).

Question 3j: You can get AIDS by having sexual intercourse with someone who has shared drug needles. ($p = 0.000$; $p < 0.01$).

Question 3k: Birth control pills protect a woman from getting the AIDS virus. ($p = 0.000$; $p < 0.01$).

In the second set of questions students were asked to state if they can have a conversation about AIDS, protection, and sex with friends, other, and adults.

Before the workshop, 54% stated that they can have a conversation, 44% stated they could not discuss the above issue, 9% of the 44% stated they "definitely could not" have a conversation, and 32% stated they were uncomfortable discussing the issue with parents and other adults such as relatives. After the workshop greater number of students stated that they can have a conversation on the topics above i.e. 66% after workshop versus.54% before the workshop; 34% stated that they could not discuss. 7% as opposed to 9% before the workshop stated that they definitely could not talk, thus the decrease in the percent of student not being able to talk bring small. There was a decrease in the number of students stating they were uncomfortable having a conversation about the above topics with parents and adults from 32% before the workshop to 26% after the workshop.

Concern about Acquiring AIDS or Any Other Sexually Transmitted Disease

Before the workshop, 83% stated that there was no chance of them acquiring AIDS. 60% stated there was no chance of them acquiring another sexually transmitted disease, while 66% stated not being worried at all. After the workshop, 71% believed that there was no chance of acquiring AIDS as compared with 82% stating that before the workshop, this was not an expected result. 57% stated they were not worried. This shows a slight decrease in concern among teenagers compared to before the workshop.

Responses to the Basic Questions Pertaining to Condoms Such as How Protective and Efficient Condoms are were Posed to Determine Level of Understanding

Before the workshop, 32% of the students answered the questions correctly. Majority of the students answered "probably would/would not" as the answer signifying that they were not completely sure of the answers. After the workshop, 38% of the answers were answered correctly, though unexpectedly there was an increase compared to the

results before the workshop, it was, however, not statistically significant. Majority still put in "probably would/would not" as the answer. The responses to the different questions pertaining to condoms are shown in Table 2.

Knowledge of Condoms, Average of Correct and Wrong

Table 1: Responses to questions 3a-3j about general knowledge of AIDS. Options were: True, False, Don't know

#3 (Q's on AIDS)	Before workshop Correct answer %	Before workshop incorrect answer %	Before workshop Don't know answer %	After workshop Correct answer %	After workshop incorrect answer %	After workshop Don't know answer %
3a. Only people who look sick can spread the AIDS virus.	31	18	51	78	8	14
3b. Condoms reduce the risk of getting the AIDS virus. (A condom is a piece of rubber that fits over the penis.	60	21	19	72	20	8
3c. A person can get the AIDS if he or she has sexual intercourse just one time without a condom.	50	34	16	61	28	11
3d. A person can get AIDS by touching or hugging someone with AIDS	56	29	15	86	10	4
3e. Most people who have the AIDS virus show signs of being sick right away.	14	82	4	30	64	6
3f. You can get AIDS by having anal sex without a condom (By anal sex we mean putting a penis in another person's anus [butt].)	54	32	14	68	30	2
3g. You can get AIDS by being bitten by a mosquito that has bitten someone with AIDS.	52	8	38	92	0	8
3h. Only people who have sexual intercourse with gay (homosexual) people get AIDS.	34	10	56	62	22	16
3i. You can get AIDS from kissing someone who has AIDS.	31	44	15	92	0	8
3j You can get AIDS by having sexual intercourse with someone who has shared drug needles.	15	28	56	68	15	18
3k Birth control pills protect a woman from getting the AIDS virus.	5	19	76	56	32	12

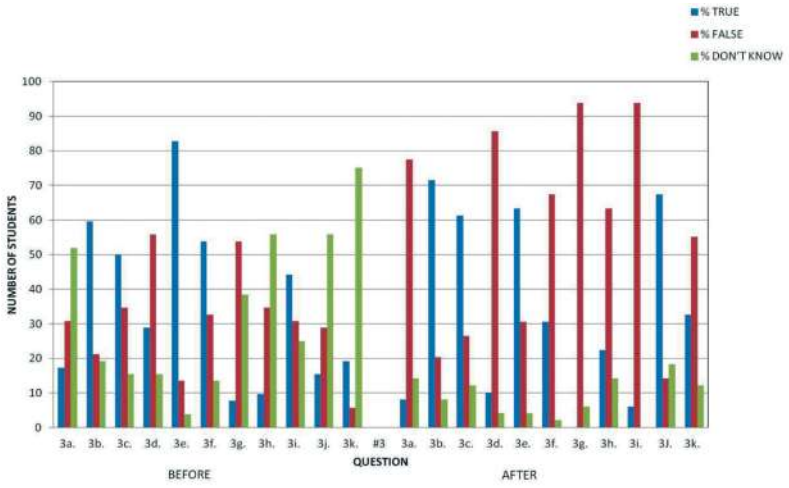

Fig. 1: Knowledge of AIDS among teens before workshop (Left side) and after workshop (right side)

Table 2: Responses to questions related to condoms before and after workshop

#12, 13, 14, 16 (Q's related to condoms)	Before: I definitely would refuse (%)	Before: I probably would refuse (%)	Before: I probably would not (%)	Before: I definitely would not (%)	After: I definitely would refuse (%)	After: I probably would refuse (%)	After: I probably would not (%)	After: I definitely would not (%)
12a. I would refuse to have sexual intercourse without c.	5.77	17.31	55.77	21.15	19.23	36.54	30.77	7.69
12b. would insist on using a condom even if my partner didn't want to.	1.92	13.46	63.46	21.15	1.92	32.69	48.08	11.54
13a. If the person I was about to have sex with suggested using condom I would feel that person cared for me	9.61	13.46	55.77	21.15	5.77	34.61	38.46	15.38
13b. If the person I was about to have sex with suggested using a condom, I would feel less worried.	17.31	23.08	32.69	26.92	28.85	32.69	23.08	9.61
13c. would respect my partner if he/she suggested condom.	5.77	36.54	32.69	25	13.46	38.46	19.23	23.08
14a. It would really bother me to stop having sexual intercourse to put on a condom.	5.77	9.61	28.85	55.77	3.85	13.46	44.23	32.69
14b. Condoms would be too much trouble to use.	7.69	13.46	21.15	59.61	7.69	17.31	28.85	40.38
14c. would **not** feel good to use it during sexual intercourse	0	28.85	48.08	5.77	0	28.85	48.08	5.77
14d.would is embarrassed to buy condoms.	0	17.31	25	57.69	0	13.46	28.85	51.92
16a. I'm worried about catching AIDS, so I would be sure to use a condom, even in the heat of the moment.	13.46	55.77	26.92	3.85	17.31	63.46	13.46	0
16b. If I didn't have a condom, I would have sexual intercourse anyway.	1.92	71.15	15.38	30.77	1.92	51.92	26.92	13.46
16c. I would use a condom even if I were drunk, high.	11.54	28.85	51.92	7.69	11.54	36.54	42.31	3.85

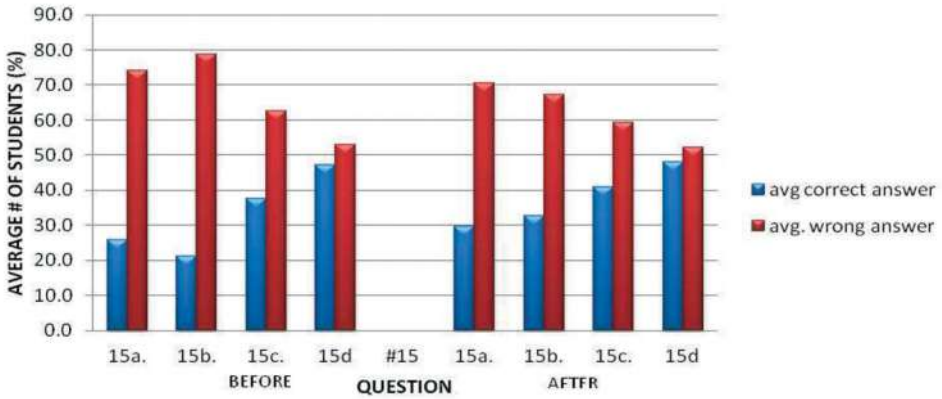

Fig. 2: Average of don't know, correct and wrong answers before and after workshop

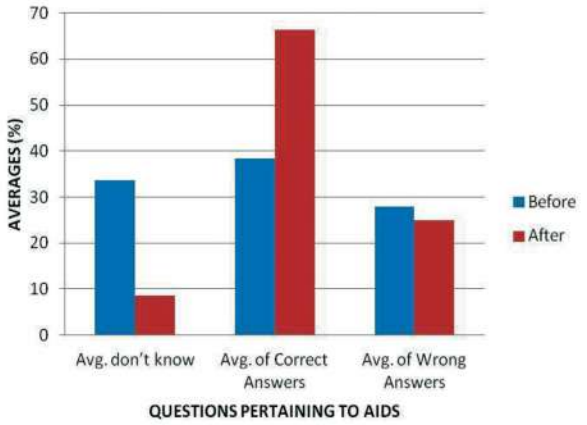

Fig. 3: Knowledge of condoms among teens before and after workshop

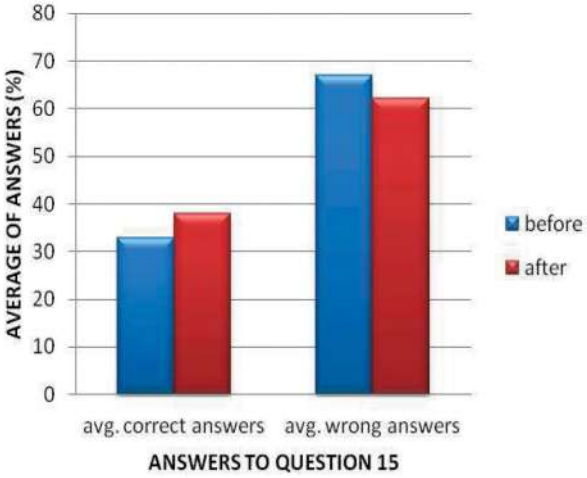

Fig. 4: Answers to Question 15 on knowledge of condoms

Answers before and after Workshop

Before the workshop, on average, 83% believed condoms would not cause trouble and that they would not be bothered by putting them on while having intercourse. 48% stated it might not "feel good" to wear a condom during sexual intercourse. After the workshop, there was a slight increase in the number of students that believed wearing condoms would cause trouble. Also there was slight increase in the number who believed it might not "feel good" to wear a condom.

Discussion

This is the first study of its kind in Bonaire and possibly also in the Dutch Caribbean. It is apparent from the results of the study that the knowledge of the school students about AIDS significantly increased after conduction of the workshop as compared to that before the workshop. Their openness for conversation about AIDS, it protection, and sex with friends, adults, and others in the social community also significantly increased after workshop as compared to that before the workshop. It is also noteworthy that their concern about acquiring AIDS or any other sexually transmitted disease slightly decreased compared before and after workshop. A reason for vulnerability of adolescents to STDS including AIDS is the lack of sex education on HIV/AIDS [11]. Sex education in schools has been considered as a 'social vaccine' and it can serve as an important powerful tool for prevention of AIDS [12]. In India a wide gap was observed between the inputs on HIV/AIDS in the curriculum of sex education and its actual implementation. In a study from India [13] a significant proportion of secondary school students demonstrated adequate knowledge of modes of transmission of HIV/AIDS; 92.1% of them stated that it was transmitted through unprotected sex, and 75.8% answered from mother to child transmission. In another study form India [14], 90.7% of the students stated sexual route while 96.6% named sharing of syringes and needles as a mode of transmission. A study from Ghana [15] revealed that senior high school girl students were generally knowledgeable on the nature, modes of transmission, and prevention of among HIV/AIDS, This may be due to the educational initiative for awareness of AIDS launched by Ghana AIDS Commission and National AIDS/HIV control program over more than the past decade. It may also be mentioned that in a study from Namibia [16], 93% of university students and polytechnic students exhibited a good knowledge of HIV/ AIDS, and 92% of all respondents knew the protective value of condoms against HIV infection.

In our study, knowledge of condoms as a means of protection did not increase appreciably after the educational effort. Analysis of results revealed that after the workshop, there was only a slight decrease in the number of students saying that they would "feel good" to wear a condom. The reluctance of senior high school girls to use condoms as a preventive measure has also been pointed in an earlier study of awareness of AIDS in senior high school students in Ghana [15]. An evidence-based study by Weller & Davis [17] has shown that using condoms consistently effectively reduces sexual transmission of HIV. Thus it is imperative to educate the youth in Bonaire and other islands in the Dutch Caribbean about the benefits of using condoms.

Conclusion

It is apparent from our study that creating knowledge and awareness on HIV/AIDS among adolescents in Bonaire is crucial not only in preventing the spread of HIV infection but also for addressing the threats posed by HIV/AIDS to the cause of education. In particular the wrong perception of the students regarding condoms needs to be corrected by educating them that condoms do not cause any trouble and there is nothing to feel embarrassed to wear them and they should feel comfortable.

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Malaria-Need for addressal and Future Challenges

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Abstract

Malaria is a major public health problem around the world. The recent trend shows a reduction in incidence and deaths due to malaria. However it is still a major concern in many countries. World Health Organization (WHO) has framed post-2015 global technical strategy on malaria with newer targets and milestones that have to be achieved by 2020, 2025 and 2030. Universal access to prevention, diagnosis and treatment and effective surveillance are some of the pillars. Government of India launched National Vector Borne Control Program (NVBDCP) in 2005 to consolidate the efforts for prevention and control of vector borne diseases in India. Recently, National malaria strategic plan for malaria control in India, 2012-17 has been framed with revised targets and strategies. To ensure success of these plans and strategies, major hurdles like lack of human resource and funds, limited private sector involvement and lack of political leadership has to be overcome.

Keywords: Malaria; Initiatives; Challenges.

Introduction

Malaria is a protozoal infection caused by four species of the genus Plasmodium (P vivax, P falciparum, P ovale and P malariae) and is transmitted by the female anopheles mosquito. It has been a major problem claiming thousands of lives every year in almost all parts of the world. Malaria has serious implications on health of people. It causes significant financial burden and reduced productivity for the affected population. Looking at the diversity of its distribution and presentation, there has been a recent resurgence in interest and activities for its prevention, control and research by the international community [4].

A number of institutions, policies and guidelines have been created to plan, implement and monitor various strategies for its prevention and control. Reduction in morbidity and mortality due to mosquito borne diseases is important to meet the overall objective of Millennium Development Goals (MDG) and National Health Policy [2]. This article discuss current trend of malaria and newer initiatives that has been taken for malaria prevention and control at national and international level.

Current Status of Malaria

Burden

As per latest estimates, about 3.2 billion people are at risk of malaria in 97 countries. In the year 2013, the disease killed about 584,000 people mostly children aged under 5 years in sub-Saharan Africa [3]. In the year 2015, there were 214 million new cases of malaria worldwide with 438 000 deaths [4]. While Africa accounts for 90% of the mortality burden for malaria, South-East Asia accounts for 9% of the burden. Out of 11 countries of the World Health Organization South East Asia Region (WHO SEARO), 10 countries are malaria endemic. Morbidity of malaria comprises severe anaemia especially in children and pregnant women, greater prevalence of low birth-weight and development anomalies from residual effects of cerebral malaria. Besides, irregular attendance at school, impaired intellectual development, reduced productivity are some of its indirect effects [5,6].

India is particularly vulnerable to malaria due to epidemics and seasonality. Malaria is concentrated more in rural areas of eastern and north-eastern states. Central and more arid western parts of the

country are also important foci. About 95% population in the country resides in malaria endemic areas and 80% of malaria reported in the country is confined to areas where 20% of population reside in tribal, hilly, hard-to-reach or inaccessible areas [7].

Trend in Malaria Situation

Between 2001 and 2013, a substantial expansion of malaria control strategies globally, averting an estimated 4.3 million deaths. In the WHO African Region, the malaria mortality rate in children under 5 years of age was reduced by 58% [3]. There has been 37% decrease in malaria incidence globally between 2000 and 2015. There is 60% estimated decrease in global malaria deaths between 2000 and 2015 [4]. Target of Millennium Development Goal 6, namely "Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases", has already been reached, and 55 of the 106 countries that had malaria transmission in 2000 have almost achieved the goal of reducing malaria incidence by 75% by 2015[8].

Newer Initiatives- International Level

A number of newer strategies and plans have been framed at international and national level. New targets have been set for coming years to accelerate the progress towards malaria prevention and control efforts.

Global Technical Strategy for Malaria 2016–2030

WHO has developed a post-2015 global technical strategy on malaria. The vision of this strategy is a world free of malaria. As part of this vision, the strategy sets global targets for 2030 with milestones for measuring progress for 2020 and 2025 as shown in Table 1. Countries can set their own national or sub national targets [8].

Table 1: Goals, milestones and targets for global technical strategy for malaria 2016-2030

Vision- A world free of malaria				
		Milestones		Targets
S. No.	Goals (taking 2015 as baseline)	2020	2025	2030
1	Reduce malaria mortality rates globally	Atleast 40%	Atleast 75%	Atleast 90%
2	Reduce malaria case incidence globally	Atleast 40%	Atleast 75%	Atleast 90%
3	Eliminate malaria from countries in which malaria was transmitted	Atleast 10 countries	Atleast 20 countries	Atleast 35 countries
4	Prevent re establishment of malaria in all countries that are malaria free	Re establishment prevented	Re establishment prevented	Re establishment prevented

The strategy has three pillars with two supporting elements to guide the efforts for malaria elimination.

1. Pillar 1 is to ensure universal access to malaria prevention, diagnosis and treatment- A number of interventions are to be promoted. Prevention strategies like vector control, chemoprophylaxis with diagnostic facilities and provision of effective treatment in every public and private health facilities should be ensured.
2. Pillar 2 is to accelerate efforts towards elimination and attainment of malaria-free status. Countries should intensify efforts to reduce the transmission of new infections in defined geographical areas. Interventions that target both vectors and parasite should be focussed. Active case finding and surveillance should be important part of efforts. Innovative technologies and strategies are essential to target the reservoirs of parasite like addressing insecticide resistance.
3. Pillar 3 is to transform malaria surveillance into a core intervention. Strengthening malaria surveillance is a fundamental requirement for planning and implementation of malaria control program. All countries should have an effective health management and information system (HMIS) for surveillance and monitoring of malaria cases, to detect outbreaks, to assess the impact of preventive and control interventions and to effectively use available resources.

Following indicators have been given to measure the outcome and impact.

Outcome –

1. Proportion of population at risk who slept under

an insecticide-treated net the previous night

2. Proportion of population at risk protected by indoor residual spraying within the past 12 months
3. Proportion of pregnant women who received at least three or more doses of intermittent preventive treatment of malaria while attending antenatal care during their previous pregnancy in sub-Saharan Africa only
4. Proportion of patients with suspected malaria who receive a parasitological test
5. Proportion of patients with confirmed malaria who receive first-line anti malarial treatment according to national policy
6. Proportion of expected health facility reports received at national level
7. Proportion of malaria cases detected by surveillance systems
8. Proportion of cases investigated
9. Proportion of foci investigated

Impact

1. Parasite prevalence: proportion of the population with evidence of infection with malaria parasites
2. Malaria case incidence: number of confirmed malaria cases per 1000 persons per year
3. Malaria mortality rate: number of malaria deaths per 100 000 persons per year
4. Number of countries that have newly eliminated malaria since 2015
5. Number of countries that were malaria-free in 2015 in which malaria was re-established

Major guiding principles for malaria control and prevention are also given. All countries should accelerate their efforts towards elimination of malaria through variety of interventions which can be modified according to local burden and available resources. Government should lead the efforts with active involvement of communities and other stakeholders. Inter-sectoral coordination is essential to guide the efforts. Improved surveillance, monitoring, evaluation, estimation of disease burden are some important steps required for implementation of malaria interventions. Health services should be available, accessible and affordable especially for the most vulnerable and in difficult geographical areas. Innovation with development of local appropriate technology in tools and approaches is important to enable countries to progress towards malaria.

On 1 January 2016, 17 Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development came into force. Like MDGs, they also aim to achieve development for all ages and sex. SDG 3 aspires to ensure health and well-being for all, including a bold commitment to end the epidemics of malaria and other communicable diseases by 2030. One of the targets of SDG 3 is to end the epidemics of malaria by 2030 [9].

National Level

Government of India launched National Vector Borne Control Program (NVBDCP) in 2005 to consolidate the efforts for prevention and control of vector borne diseases in India. Recently, under NVBDCP, National malaria strategic plan for malaria control in India, 2012-17 has been framed [7]. To achieve API < 1 per 1000 Population by the end of 2017 is objective of this plan. The mission statement of this plan is to reduce the morbidity and mortality due to malaria and improving the quality of life, thereby contributing to health and alleviation of poverty in the country.

Following Goals has been Set for this Plan-

1. Screening all fever cases suspected for malaria (60% through quality microscopy and 40% by Rapid Diagnostic Test)
2. Treating all P. falciparum cases with full course of effective Artemisenin Combination Therapy (ACT) and primaquine and all P. vivax cases with 3 days chloroquine and 14 days primaquine.
3. Equipping all health Institutions (PHC level and above), especially in high-risk areas, with microscopy facility and Rapid Diagnostic Tests (RDTs) for emergency use and injectable artemisinin derivatives
4. Strengthening all district and sub-district hospitals in malaria endemic areas as per Indian Public Health Standards (IPHS) with facilities for management of severe malaria cases.

Various Outcome Indicators has been Set to Monitor the Implementation

1. At least 80% of those suffering from malaria get correct, affordable and appropriate and complete treatment within 24 hours of reporting to the health system, by the year 2017
2. At least 80% of those at high risk of malaria get

protected by effective preventive measures such as ITN/LLIN or IRS by 2017

3. At least 10% of the population in high-risk areas is surveyed annually (Annual Blood Examination Rate >10%)

Following are Impact Indicators for the Plan-

1. To bring down annual incidence of malaria to less than 1 per 1000 population at national level by 2017.
2. At least 50% reduction in mortality due to malaria by the year 2017, taking 2010 level as baseline

Various strategies which have been opted in the plan are focusing on reforming the program planning and management. Improvement in surveillance and strengthening the monitoring and evaluation would be a priority. Scaling up the coverage and use of insecticide treated bed nets among populations is essential. Targeted interventions to risk groups is major provision in the plan. Use of Artemesinin Combination Therapy (ACT) and Rapid Diagnostic tests (RDTs) at village level and Integrated Vector Management (IVM) along with Long Lasting Insecticide Treated Bednets (LLIN) use is envisaged. Since incidence of malaria is progressively shrinking in India, under this plan, it is proposed to change the strategies according to malaria endemicity at state and district level.

1. For areas having perennial transmission (more than 5 months in a year) - 2 rounds of Indoor Residual Spray (IRS) with DDT/Synthetic Pyrethroids (SP) or 3 rounds with malathion.
2. For areas having seasonal transmission (less than 5 months in a year) - 1 round of IRS with DDT/ SP or malathion before start of transmission season; focal spray if needed; and priority distribution of LLINs.

For states which are reporting an API of < 1 for three consecutive years, they are to initiate action for declaring malaria as a notifiable disease in the state

Table 2: Strategies under National malaria strategic plan for malaria control in India, 2012-17

Category	Definition	Strategies
1	States with API less than 1 and all the districts in the state are with API less than 1	Active, passive and sentinel surveillance Screening of migrants Integrated vector management (IVM) with inter-sectoral coordination Behaviour Change Communication (BCC)
2	States with API less than 1 and one or more districts in the state are with API more than than 1	Surveillance and disease management (T3- test, treat and track) Screening of migrants Integrated vector management (IVM) with inter-sectoral coordination Behaviour Change Communication (BCC) with NGOs
3	States with API more than 1	Surveillance and disease management Management of severe malaria by strengthening of district and sub district hospitals and referral services Integrated vector management (IVM) by IRS and LLITNs Supportive interventions

for improved surveillance.

Core interventions and target objectives are as follows-

For Reducing Disease Burden and Mortality

Prevention using Insecticide treated mosquito nets would be priority. Objective has been set that by March 2017, 80% of population in high-risk areas sleep under an insecticide treated bed-nets. For Indoor residual spraying, it has been set that by March 2017, 85% of people living in households eligible for IRS have their homes sprayed annually.

For care and treatment, it is proposed that by March 2017, at least 80% of those suffering from malaria get correct, affordable and appropriate diagnosis within 24 hours of reporting to the health system. Also, by March 2017, at least 80% of malaria patients in high-risk areas are receiving prompt and effective treatment according to the current drug policy within 24 hours of reporting to the health system. Intersectoral coordination and public private partnership (PPP)

should be promoted.

PPP can be in form of liaison with international organizations, NGOs, private practitioners and voluntary groups to undertake activities like awareness generation, training of health workers, technical guidance and funding.

Future Challenges

Shortage of trained manpower and finances will be a major challenge. A number of posts of entomologist, health assistant/supervisors (Male), malaria inspectors and assistant malaria officers and field staff are lying vacant. Lack of money for take up interventions at community levels is a main hurdle. Similarly lack of rapid response teams for outbreak investigations has to be overcome.

Resources available for diagnosis and medicines need to be streamlined so that they are available in all health facilities. Monitoring and evaluation need to be strengthened at all levels. Private sector involvement remains limited.

The above mentioned challenges have to be overcome to meet the targets for malaria control in future. Political will is important to take leadership. Continues efforts are required for strengthening the malaria control efforts.

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Combact: Hepatitis A

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Abstract

The term viral hepatitis refers to primary infection of the liver by any one of a heterogeneous group of hepatitis viruses which is currently consist of types A,B,C,D,E and G. Type A hepatitis (infectious hepatitis)is a sub acute disease of global distribution, affecting mainly children and young adults. The large majority of infectious are asymptomatic. Over illness is seen in only about 5percent of those infected. Natural infection with HAV, clinical or subclinical leads to lifelong immunity. There is no cross immunity between HAV and any of other hepatitis viruses. Treatment is asymptomatic .no specific antiviral drug is available.

Keywords: Hepatitis; Treatment; Vaccination; Infection; Immunity; Viremia.

Introduction

Every year on July 28, WHO world hepatitis day to provide the awareness and understanding regarding viral hepatitis and the disease that it causes. Viral hepatitis a group of infectious disease known as hepatitis A, B, C, D, E and G affects hundreds of millions of people worldwide, causing acute and chronic liver disease and killing close to 1.4million people every year. But hepatitis remains largely ignored or unknown. On world hepatitis day July 28, WHO and partners will urge policymakers, health workers and the public to 'THINK AGAIN' about this silent killer.

World hepatitis day provides an opportunity to focus on specific action such as:

- Strengthening prevention, screening and control of viral hepatitis and its related disease;
- Increasing hepatitis B vaccine coverage and integration of the vaccine in to national immunization programme;
- Coordinating a global response to viral hepatitis.

Hepatitis A typically caused by ingestion of contaminated food and water is primarily spread when someone who has never been infected with hepatitis A and is not vaccinated, ingests food or water that is contaminated with the feces of an infected person. Hepatitis A does not cause chronic liver disease and is rarely fatal, but it can cause serious symptoms.

Definition

Hepatitis A is a highly contagious liver infection caused by the hepatitis A virus. The virus is one of several types of hepatitis viruses that cause inflammation and affect the liver's ability to function.

History

Hepatitis A is referred to as one of the oldest disease known to mankind by the WHO. It was recognized by as a separate entity from other types of hepatitis during world war II. Hepatitis A was discovered in 1973 by Steven's, Feinstone as a non enveloped, spherical, positive stranded RNA virus. HAV was an unidentified viral disease prior to this discovery.

Incidence

Globally, around 1.5 million symptomatic cases occur each year and there were about 102 million cases in 2013. It is more common in regions of the world with poor sanitation and not enough safe water. In the developing world, about 90% of children have been infected by age 10, thus are immune by

adulthood. It often occurs in outbreaks in moderately developed countries where children are not exposed when young and vaccination is not widespread. In 2010, acute hepatitis A resulted in 102,000 deaths.

Structure of the HAV

Hepatovirus A is a picornavirus; it is non enveloped and contains a single stranded RNA packaged in a protein shell. There is only one serotype of the virus, but multiple genotypes exist. Codon use within the genome is biased and unusually distinct from its host. It also has a poor internal ribosome entry site. In the region that codes for the HAV capsid, highly conserved clusters of rare codons restrict antigenic variability.

Etiology of Hepatitis A

Anyone can get hepatitis A, but those more likely to affect people who:

- Travel to developing countries.
- Live with someone who currently has an active hepatitis infection.
- Overcrowding.
- Poor sanitation.

Transmission of Hepatitis A

Transmission of hepatitis A through contact with an infected person's stool. This contact could occur by,

- Eating food made by an infected person who didn't wash his or her hands after using the toilet.
- Drinking untreated water or eating food washed in untreated water.
- Placing a finger or object in mouth that came in to contact with an infected person's stool.
- Infected flies

Pathogenesis of Hepatitis A

Following ingestion of contaminated food, HAV enters the bloodstream through the epithelium of the oropharynx or intestine. The blood carries the virus to its target, the liver, where it multiplies within hepatocytes and Kupffer cells (liver macrophages). Viral replication is cytoplasmic. Entry into the host cell is achieved by attachment of the virus to host receptors, which mediates endocytosis. Replication follows the positive-stranded RNA virus replication model. Positive-stranded RNA virus transcription is the method of transcription. Translation takes place by viral initiation. The virus exits the host cell by lysis, and viroporins. Virions are secreted into the bile and released in stool. HAV is excreted in large quantities about 11 days prior to appearance of symptoms or anti-HAV IgM antibodies in the blood.

Incubation Period

15-50 days

Symptoms of Hepatitis A

- Extream fatigue
- Muscle soreness
- Upset stomach
- Fever
- Loss of appetite
- Stomach pain
- Nausea and vomiting
- Diarrhea
- Dark-yellow urine
- Light colored stools
- Yellowish eyes and skin, called jaundice.

Diagnosis of Hpatitis A

Blood Test

- IgG.The presence of IgG antibodies in the blood means the acute stage of the illness is past and the person is immune to further infection.
- HAV- specific IgM Antibodies in the blood. IgM antibody is only present in the blood following an acute hepatitis A infection.
- ALT. During the acute stage of the infection, the liver enzyme AlanineTtransferase (ALT) is present in the blood at levels much higher than

is normal. The enzyme comes from the liver cells damaged by the virus

Stool Examination

- Hepatovirus A is present in the blood (viremia) and feces of infected people up to two weeks before clinical illness develops.

Treatment of Hepatitis A

There is no specific treatment for hepatitis A. Recovery from symptoms following infection may be slow and may take several weeks or months. Therapy is aimed at maintaining comfort and adequate nutritional balance, including replacement of fluids that are lost from vomiting and diarrhea.

Prevention of Hepatitis A

Health education of people,

- Environmental sanitation.
- Food hygiene.
- Use of boiled drinking water.
- Hand washing before taking food and after toilet.
- Personal hygiene in that cut short the nails.
- Avoid contamination of food and water by covering food and protection from flies.
- Avoiding infected water source or food (undercooked shell fish).
- Prophylaxis with immune globulin before or early in intubation (<2weekspost exposure) is 80-90% effective. In that Hepatitis A vaccine (inactivated) prenatal administration 2 dose regimen, 6-18 months apart.
- Children: Hep A + Recombinant Hep B.
- Vaccination 0,1,6 monthsof food handlers.
- Two inactivated whole–virus hepatitis A vaccine are available; HAVRIX (GlaxoSmithKline) and VAQTA (Merck).

Prognosis

The risk of death from acute liver failure following HAV infection increases with age and when the person has underlying chronic liver disease. Young children who are infected with hepatitis A typically have a milder form of the disease, usually lasting from 1–3 weeks, whereas adults tend to experience a much more severe form of the disease.

Conclusion

Hepatitis A is a preventable misery to mankind. Hepatitis A is primarily spread when someone who has never been infected with hepatitis A and is not vaccinated, ingests food or water that is contaminated with the feces of an infected person or has direct contact with someone who is infected. Hepatitis A does not cause chronic liver disease and is rarely fatal, but it can because serious symptoms Health educate and follow the hygienic practices, we can fight with this disease from our community.

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Dengue Fever

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Abstract

Dengue virus is an acute febrile viral condition, is not transmitted from person to person but through the bite of an infected mosquito and it is caused by Flavivirus.Main symptom of dengue fever is body temperature more than 38°C, body pain and joint pain. In dengue fever Platelet count will decrease. Prevention of dengue fever is by Insecticide treatment and symptomatically manages the dengue fever.

Keywords: Dengue; Fever; Immunity; Mosquito; Shock; Vaccine.

Introduction

In the present scenario, where non-communicable disease is getting prime importance, there are some communicable diseases which take over the picture and affect a greater community and cause complications. Very many communicable diseases have a vector-mosquito, which pass on the disease. One of such vector-borne disease which is getting through the attention is dengue fever. Dengue is an acute viral infection with potential fatal complications. It was first referred as "water poison" associated with flying insects. The word "dengue" is derived from the Swahili phrase Ka-dinga pepo, meaning "cramp-like seizure". Dengue fever is an arthropod borne virus of the genus Flavivirus, and within the family Flaviviridae. Other flaviviruses include Japanese encephalitis and yellow fever.

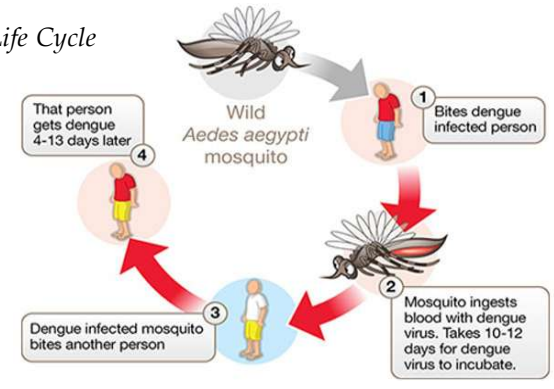
History

Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice3 and at Calcutta (now Kolkata) in 1944 from serum samples of US soldiers. The first epidemic of clinical dengue like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964. The first major epidemic of the DHF occurred in 1953-1954 in Philippines followed by a quick global spread of epidemics of DF/DHF. DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in various parts of India since 1989. The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi and Luck now and then it spread to all over the country.

Genome Structure of Dengue Virus

Its genome is about 11000 bases that codes for there structural protein [Capsidprotein C, membrane protein M, Envelope protein E] and seven nonstructural protein [NS1, NS2a,NS2b,NS4a, NS4b,NS5a,NS5b]. It also includes short noncoding regions on both the 5′ and 3′ ends. Further classification of each serotype into genotypes often relates to the region where particular strains and commonly found or were first found.

Life Cycle



Epidemiology

In World

Today about 2.5 billion people, or 40% of the world's population, live in areas where there is a risk of dengue transmission. Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. The World Health Organization (WHO) estimates that 50 to 100 million infections occur yearly, including 500,000 DHF cases and 22,000 deaths, mostly among children.

In India

With 19,704 cases reported till September 6, the dengue cases in the country have already doubled this year. In 2014, the number of dengue cases stood at 10,097, with 37 deaths, through the year.

This year, however, 41 people had died, though the Health Ministry said in a statement that with a fatality rate of 0.20 per cent, casualties remained "very low". The highest fatality rate reported in India so far was during the 1996 outbreak, when it was more than 3 per cent.

In Kerala

Dengue fever was first reported in Kerala in 1997 in Kottayam district. First epidemic occurred in 2003 with 3546 cases and 68 deaths. Thiruvananthapuram was the worst affected district.

Dengue fever has become endemic in Kerala.in the year of 2011 with 1287 cases and 10 deaths has been reported. Officials point out that all figures show that there has been a steady increase in Dengue cases which may shoot in the three months when the state receives monsoon rains.

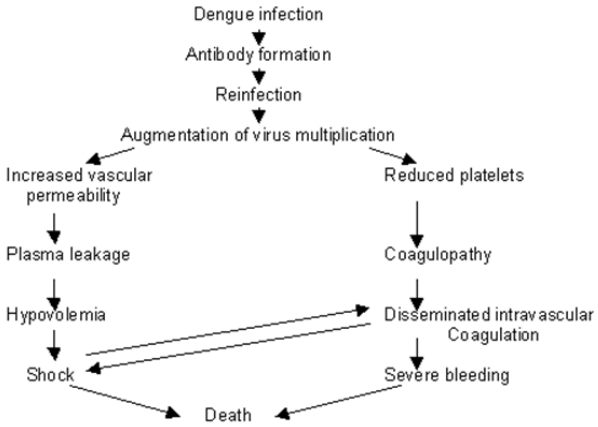
Mode of Transmission

Transmission cycle in dengue "Man to mosquito,Mosquito to man"

The vector Aedes argypti acquires the virus by feeding on a patient during the first 3 days [viralmic stage] of illness. After the period of 10-15 days the mosquito becomes infective and is able to transmit the infection to man.

Incubation Period

Incubation period in 4-10 days after bite of the mosquito.



Pathophysiology

Types

1. Dengue Viral fever (DUF)
2. Dengue Hemorrhagic fever (DHF)
3. Dengue shock syndrome(DSS)

Clinicalmanifestations

Dengue Fever (1-4 days)

- Body temperature more than 38 ^{o}C, which lasts up to 5-7 days.
- Headache and pain diretro-orbital (behind the eye).
- Pain in muscles and joints.
- Nausea and vomiting, loss of appetite.
- The presence of digestive disorders (constipation or diarrhea).
- Abdominal pain.
- The presence of rash (signs of redness) of the skin.

Dengue Hemorrhagic (4-7days)

- Spontaneous bleeding.
- Organ enlargement of the liver (liver) and spleen.
- The presence of thrombocytopenia, the platelet count is less than 100.000/mm^{3}.
- Plasma leakage marked with hematocrit values is increased or decreased by 20% or more of normal values.
- Pleural effusion and ascites.

Dengue Shock Syndrome (DSS)

- An impairment of consciousness.
- Very low blood pressure.

- Rapid and weak pulse.
- Hands and feet pale and cold.

The WHO, Divide into 4 Degrees of Clinical Manifestations,

- *DHF Grade I:* The signs of viral infection, the manifestation of bleeding that seemed only to test positive torniquet.
- *DHF Grade II:* Signs manifestations of viral infections with spontaneous bleeding (nosebleeds, red spots)
- *DHF Grade III:* Also called pre-shock phase, with signs of DHF grade II but the patient began to experience signs of shock; decreased consciousness, cold hands and feet, rapid and weak pulse palpable, pulse pressure was measured.
- *DHF degree IV:* Or the phase of shock (dengue shock syndrome also called / DSS), patients in shock with greatly decreased consciousness and coma, cold hands and feet and pale, the pulse is very weak to not palpable, pulse pressure can not be measured.

Diagnostic Tests

History Collection &Physical Examination

Lab Values

- *Platelet Count*

Value become less than 1, 00,000 cells/mm^3

- *Hematocrit Value*

Is increased by 20% or more The clinical criteria like high fever, spontaneous hemorrhagic manifestation associated with thrombocytopenia and rise in hematocrit value are sufficient to establish the diagnosis of DHF. Hypoproteinemia, pleural effusion and ascites constitute the supporting evidence of plasma leakage.

- *Real Time Polymerase Chain Reaction*

This is done to detect viral genome in serum. It is primary tool to detect virus early in the course of illness. It is a definite proof of current infection. But this test is not available.

- *NSI ELISA Test*

Detection of non structural protein (NSI antigen) in the serum of dengue fever patients is an useful tool for the diagnosis of acute dengue infections this is commercially available.

- *IG GELISA Test*

Samples with negative IgG in acute and positive IgG in convalescent phase of the infection are primary dengue infection. Sample with a positive IgG in the acute phase and a four fold rise in IgG titre in the convalescent phase, is secondary dengue infection.

- *Plaque Reduction and Neutralization Test*

The most specific serological tool for the determination of dengue antibodies is plaque reduction and neutralization test assay. This determines the level of antibodies.

Prevention

Personal

- Clothing to reduce exposed skin
- Insect repellent especially in early morning, late afternoon. Bed netting important
- Mosquito repellants(pyrethroid based) coils, sanitation measures

Environmental

- Reduced vector breeding sites
- Solid waste management
- Public education
- Empty water containers and cut weed/tall grass

Biological

- Target larval stage of Aedes in large water storage containers
- Larvivorous fish (Gambusia), endotoxin producing bacteria (Bacillus), copepod crustaceans (mesocyclops)

Chemical

Thermal fogging-malathion,pyrethrum

- Insecticide treatment of water containers
- Space spraying (thermal fogs)
- Indoor space spraying(2% pyrethrum), organophosphorus compounds.

Management

Antiviral Drugs

There are no specific antiviral drugs for dengue.

Oral Rehydration Therapy

However maintaining proper fluid balance is important. Treatment depends on the symptoms. Those who are able to drink, are passing urine, have no "warning signs" and are otherwise healthy can be managed at home with daily follow up and oral rehydration therapy.

Intravenous Hydration

If required, is typically only needed for one or two days. In children with shock due to dengue a rapid dose of 20mL/kg is reasonable. The rate of fluid administration is than titrated to a urinary output of 0.5–1 mL/kg/h, stable vital signs and normalization of hematocrit. The smallest amount of fluid required to achieve this is recommended.

Paracetamol

Acetaminophen is used for fever

NSAIDs

Discomfort while NSAIDs such as ibuprofen and aspirin are avoided as they might aggravate the risk of bleeding.

Blood Transfusion

Is Initiated early in people presenting with unstable vital signs in the face of a decreasing hematocrit, rather than waiting for the hemoglobin concentration to decrease to some predetermined "transfusion trigger" level

Recovery Phase

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload. If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, a loop diuretic such as furosemide may be used to eliminate excess fluid from the circulation

Vaccine

No vaccine is currently approved for the prevention of dengue infection. Because immunity to a single dengue strain is the major risk factor for dengue hemorrhagic fever and dengue shock syndrome, a vaccine must provide high levels of immunity to all 4 dengue strains to be clinically useful.

Immunogenic, safe tetravalent vaccines have been developed and are undergoing clinical trials. Candidate vaccines include a live-attenuated virus, recombinant envelope proteins, and an inactivated virus. The estimates of the time needed for further testing of candidate vaccines range from 5-10 years. Sanofi Pasteur has reported successful results of phase II trials of its tetravalent recombinant live attenuated vaccine. Registration is anticipated in 2012.

Nursing Management

- *For Hemorrhage* – Keep the patient at rest during bleeding episodes. For nose bleeding, maintain an elevated position of trunk and promote vasoconstriction in nasal mucosa membrane through an ice bag over the forehead.
- *For Melena*- Ice bag over the abdomen. Avoid unnecessary movement. If transfusion is given, support the patient during the therapy. Observe signs of deterioration (shock) such as low pulse, cold clammy perspiration, prostration..
- *For Shock* – Prevention is the best treatment. Dorsal recumbent position facilitates circulation.
- Adequate preparation of the patient, mentally and physically prevents occurrence of shock.
- Provision of warmth-through lightweight covers (overheating causes vasodilation which aggravates bleeding).
- Diet – low fat, low fiber, non-irritating, non-carbonated.

Complication

- High fever
- Damage to the lymphatic system
- Damaged to the blood vessel
- Bleeding from the gums
- Bleeding from the nose
- Liver enlargement
- Circulatory system failure

Conclusion

Dengue disease continues to involve newer areas, newer populations and is increasing in magnitude, epidemic after epidemic. Every aspect of dengue viral infection continues to be a challenge; the pathogenesis of severe dengue disease is not known, no vaccine is yet available for protection and the vector

control measures are inadequate. Even though dengue virus was isolated in India in 1944, but the scientific studies addressing various problems of dengue disease have been carried out at limited number of centres. Though clinical studies have reported on dengue disease in India, but these are largely based on diagnosis made by kits of doubtful specificity and sensitivity. A lot more remains to be achieved for creating an impact.

Acknowledgement

Praise and glory to the lord almighty who is source of strength and inspiration in every passes in my life and foundation for the knowledge and wisdom. I extend my sincere gratitude to prof. Nandini M Vice Principal and Mrs.Ansa C J, Assisst.professor. Aswini college of nursing, for giving me proper guidance to write the scientific paper.

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Is There Hidden Wastage of Anti-Rabies Vaccine and Rabies Immunoglobulinsin India Due to Excess Vaccination?

Suneela Garg*, Saurav Basu, Archana Ramalingam*****

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Abstract

Rabies is a major public health challenge in India with the highest burden globally. Anti rabies vaccine (ARV) and anti rabies serum (ARS) provide safe and effective post exposure prophylaxis (PEP) in animal bite cases. However, providing universal PEP to incident animal bite cases is a major challenge in resource constrained settings especially due to the high cost of vaccine and serum. Hence, prevention of vaccine wastage is indispensable for rabies control in the developing world. Guidelines for rabies prophylaxis in incident animal bite cases with previous history of exposure and associated PEP recommend a two dose booster regimen on day 0 and 3. Nevertheless, evidence from some socio-epidemiological studies on rabies reported from India suggest that there exist several patient and provider related factors which promote an unwarranted universal repetition of complete course of rabies PEP in re- exposure to animal bite cases. This potentially results in an avoidable wastage of 2 doses of ARV and/or ARS per animal bite case who have previously received full course of rabies PEP. Animal bite victims with low socioeconomic and educational status have greater likelihood of being subject to excess rabies PEP vaccination. This is often due to their inability to produce related medical records and lack of awareness of the treatment received during past animal bite exposure. Lack of knowledge relating to postexposure schedule in previous vaccinated cases among residents and medical graduates may also contribute to rabies vaccine wastage.

Keywords: Rabies; India; PEP; Vaccine Wastage; Vaccination.

Rabies is a major public health challenge due to its 100% fatal character. Although, modern anti rabies vaccines and anti-rabies serum during post exposure prophylaxis (PEP) provide complete protection against rabies, the costs associated with vaccination are a major barrier in controlling rabies in developing nations [1]. India has the highest burden of rabies mortality in the world with almost 20,000 rabies cases reported annually [2] which is disconcerting since PEP for rabies after animal bite is available free of cost in most government hospitals and community health centers. Patient related factors which impede adoption of PEP against rabies in animal bite victims have been identified as ignorance of rabies especially among those of a low socioeconomic status, fear of multiple painful injections and the long duration of the treatment involved [3]. Healthcare system related factors which hinder universal PEP in animal bite victims are the inconsistent supply and lack of availability of either anti rabies vaccine (ARV) or rabies immunoglobulins (RIG). The long term goal for reduction of cost of burden on the healthcare system includes immunization of stray dogs, pre exposure prophylaxis of vulnerable populations like children in slums where human animal interaction is common and ultimately reduction in the incidence of dogbites [4,5]. However, there is a question of feasibility in attainment of these objectives in the short term. The reduction in the costs involved in universal PEP coverage with ARV and ARS in all animal bite cases with potential for rabies transmission is hence necessitated through improved, cost effective approaches like the promotion of the use of intradermal anti rabies vaccines in urban areas. We suggest that there may be an additional but

overlooked modality for reduction in vaccine wastage by adherence to rabies vaccination guidelines and preventing excess vaccination among previously vaccinated persons.

Globally, Rabies prophylaxis guidelines state that among incident animal bite cases with a positive history of a previous animal bite exposure those having previously received full post-exposure treatment with a potent cell-culture vaccine should be given only two booster doses, intramuscularly / intra-dermally on days 0 and 3, but rabies immunoglobulin is not necessary [6-8].The only exception to this guideline are patients who are immunocompromised due to HIV/AIDS or other causes which may cause loss of immunological memory and who consequently should be administered full PEP inclusive of four doses of ARV.

This implies that providing rabies PEP with a complete course containing four doses of the ARV (0,3,7,28) in a non-immunocompromised incident animal bite case with a previous history of animal bite exposure and complete PEP causes excess vaccination and consequent wastage of two doses of the vaccine. Similarly, a repeat RIG dose in an animal bite case who has previously received RIG prophylaxis may also be unwarranted. The reasons for suchpotentially excess vaccination may be attributed to both patient and provider related factors. Patient related factors are associated with the low socioeconomic status of almost three fourths of India's animal bite victims [9]who often lack awareness of the disease, are unable to differentiate specific rabies prophylaxis treatment from supportive care involving multiple injection treatment and may be unable to preserve medical records relating to rabies prophylaxis due to carelessness, frequent migration, frequent water logging of their homes. A community based study in rural and urban slums of Delhi by Sharma et al (2016) found most dog bite victims lacking any records for past immunization and few were aware that ARV was specifically given for protection against rabies [10]. Healthcare providers may also lack adequate knowledge for management of animal bite patients. Some studies have found significant proportion of medical interns and residents having inadequate levels of knowledge of appropriate animal bite management with rabies PEP [11-12]. In the study by Garg et al (2013) in Delhi, only 40.4% of allopathic doctors knew the correct postexposure schedule in previously vaccinated animal bite cases [14]. Moreover, in our observation, healthcare providers usually do not restrict rabies PEP in animal bite cases with a history of previous animal bite exposure probably associated with rabies PEP without production of relevant medical records. This is particularly due to the lethality of the disease and providers lacking confidence in accepting verbal histories from patients affirming reception of rabies PEP treatment especially when advanced by those who are illiterate or lack functional literacy skills. Moreover, the immune status of the animal bite cases is often not known to the healthcare provider.

Unfortunately, an accurate estimation of the magnitude of such excess vaccination in animal bite re-exposure cases is hampered by the lack of epidemiological data since previous history of animal bite exposure and treatment availed among new incident animal bite cases has been sparsely reported in studies from India. However, a study by Jain et al (2014) among incident cases of dog bite reporting for treatment at the OPD of the community health center at Muradnagar, Ghaziabad found 28% of the patients reporting history of previousdog bite exposure [15]. Most epidemiological studies among animal bite cases conducted in India have reported class II and class III patterns of bites among majority of the animal bite victims which mandates provision of anti-rabies serum to them [10, 11,16]. Extrapolating this evidence would tentatively mean that for every 100 dog bite patients reporting to government health centers for rabies PEP, up to 28 cases could probably have history of previous animal bite exposure with associated PEP. Assuming the lack of availability of rabies PEP based medical records during the previous episode of animal bite exposure in such cases could potentially result in excess vaccination with 56 doses of ARV and 28 doses of RIG in defiance of national guidelines for rabies PEP in animal bite victims.

In terms of costs based on prevalent market rates of ARV and RIG, a tentative estimate would amount to INR 18,872 and INR 12,600 on ARS and ARV respectively per 100 animal bite cases. Moreover, the uncalculated opportunity costs involved in terms of the time invested by the healthcare system staff in the excess vaccination process are likely to be quiet significant. The wages lost by the patient for two excess visits is 1.2 wage days each [9], which equates to a loss of 33.6 wage days per 100 animal bite cases. Considering animal bite incidence of 1.7% in India and only 47% receiving ARV [9], the enormous costs incurred due to such excess vaccination will be enormous and seriously undermine the goal of attaining universal PEP for all animal bite cases in India.

Future epidemiological studies among incident animal bite cases should record history of previous animal bite exposure, doses of ARV and ARS received

in the past, availability of past medical records related to the event, adherence of healthcare providers to national rabies PEP guidelines and their prescribing patterns when medical records of previous rabies PEP are unavailable in order to accurately estimate the levels of excess vaccination in animal bite victims.

Measures to contain the wastage through excess vaccination in previously vaccinated animal bite patients should include healthcare providers explaining the animal bite victims being vaccinated the necessity of preserving their medical record containing details of rabies PEP vaccination, training of healthcare providers with regard to proper management of animal bite patients with PEP especially among previously vaccinated persons and finally the meticulous maintenance of records of animal bite cases within healthcare systems rendering it possible to verify history of previous vaccination in new incident cases in case of non-availability of medical records with the patients. Furthermore, national guidelines for rabies prophylaxis should include a decision making algorithmic mechanism when the healthcare provider is confronted with a situation with potential for excess rabies PEP vaccination.

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Case Report

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Isolated Tuberculosis of the Epididymis

Rajendra B. Nerli*, Prasad V. Magdum*, Amey Pathade*, Amit M Mungarwadi*, Shivagouda M. Patil*, Shridhar Ghagane, Murigendra B. Hiremath****

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Abstract

It is generally agreed that tuberculosis of the epididymis is secondary to tuberculosis of the genitourinary tract. We report on a case of isolated tuberculosis of the epididymis in the absence of obvious renal, bladder or prostatic involvement.

Keywords: Epididymo-Orchitis; Tubercular Epididymitis; Biopsy; Histopathological Examination.

Introduction

Extrapulmonary tuberculosis (TB) accounts for about 10% of all tuberculosis cases. Genitourinary (GU) TB accounts for 30% to 40% of all extrapulmonary TB, second only to lymphonodal affection [1,2]. In developed countries, urogenital tuberculosis occurs in 2% to 10% of cases of pulmonary tuberculosis, while in developing countries it occurs in as many as 15% to 20% of cases [1]. Tubercle bacilli reach the epididymis by hematogenous spread. The disease initially affects the more vascular globus minor. Tubercles form within the epididymal epithelium eliciting a chronic inflammatory reaction that subsequently leads to fibrous narrowing and possible obliteration of the lumen. With disease progression, large caseous foci may form leading to a nodular epididymis [1].

It is generally believed that tuberculosis of the epididymis is secondary to tuberculosis elsewhere in the genitourinary tract. At times tuberculous epididymo-orchitis may be the first or only presentation of GU tuberculosis. It is also well known that intravenous urogram or urine examination may fail to identify a renal lesion, therefore all cases of isolated epididymal tuberculosis may not be isolated in true sense. We report on a rare case of isolated epididymal tuberculosis in a young adult.

Case Report

A 32 year old unmarried male patient approached the urological services of the hospital with symptoms of painless scrotal swelling of one month duration. The patient was to get married and hence was worried as to whether this swelling would affect his sexual life. The patient had no other local/constitutional symptoms such as fever, loss of weight/appetite and pain. Routine urine examination showed no abnormality. Ultrasonography showed an enlarged epididymis with secondary hydrocele on the left side. The right hemiscrotum appeared normal. The rest of urinary tract appeared normal. A clinical diagnosis of chronic epididymo-orchitis was made and patient was put on a course of oral fluoro-quinolones.

The swelling persisted and Magnetic resonance imaging (MRI) revealed a bulky left epididymis with mild degree hydrocele. No areas of necrosis were noted. The patient was advised biopsy/excision of epididymis. The serum tests for HIV infection were negative and the patient had a normal chest x-ray. Testicular tumor markers were within normal limits. On exploration of the scrotum, the tunica appeared thick, the hydrocele fluid appeared hazy and the testis and epididymis appeared inflamed (Figure 1 and 2). The epididymis was firm to craggy to feel. The firm part of the epididymis was excised and sent for histopathological examination. The histopathological report confirmed the diagnosis of tuberculosis with sections of epididymis showing numerous caseating granulomatous lesions. The patient was started on anti-tubercular treatment.

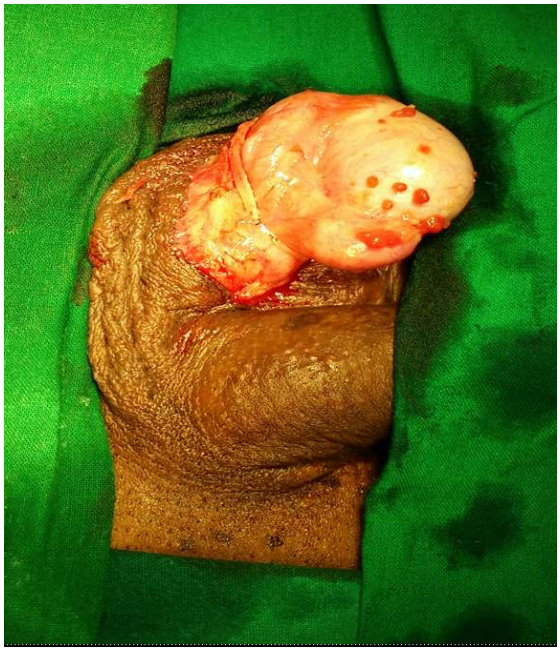

Fig. 1: Explored left testis with epididymis

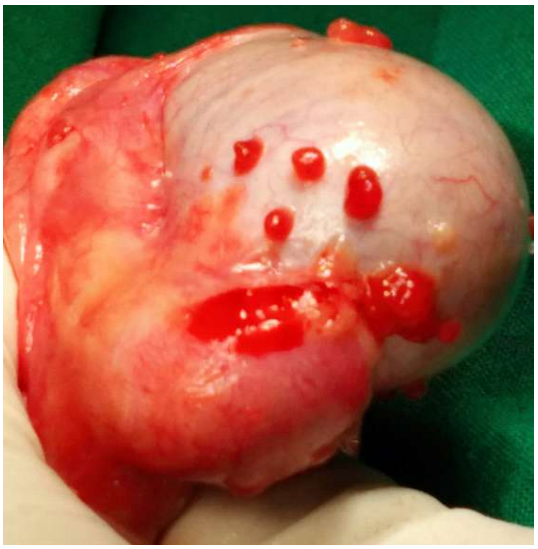

Fig. 2: Tubercles over the left testis and the epididymis

Discussion

Tubercle bacilli can affect more than one of the organs of the genitourinary tract and cause a chronic granulomatous infection. The spread of tuberculosis to the epididymis is considered to take place hematogenously or through a retro canalicular hematogenous pathway from an infected prostate. Because epididymal tuberculosis is more common than prostatic tuberculosis, the former mechanism is likely the more common one [3]. Isolated tuberculous epididymitis commonly develops in sexually active young men and is reported as the clinical onset of human immunodeficiency virus infection [4]. The epididymis can also be involved by retrograde spread of infection from the urinary bladder and/or prostate [1].

In the early phases, tuberculous epididymitis is not discernible from bacterial epididymo-orchitis. The scrotal contents are enlarged and tender, with loss of definition between the epididymis and testis. Painful or painless scrotal swelling is a common feature at presentation in patients with tuberculous epididymitis. The involvement is usually unilateral [5]. In rare cases, acute or chronic non-specific epididymitis can be confused with tuberculosis, because the onset of tuberculosis is occasionally quite painful. The presence of sterile pyuria is a useful sign of tuberculous epididymitis. If the epididymal infection is extensive and an abscess forms, it can rupture through the scrotal skin, thus establishing a permanent sinus. Alternatively, it can extend into the testis [4, 5].

A positive tuberculin test supports TB infection but a negative test does not rule it out. Polymerase Chain Reaction (PCR) facilitates and accelerates the diagnostic specificity and sensitivity of 98% and 95% respectively [1]. Although scrotal ultrasonography is helpful in the assessment of scrotal tumors, the appearance of epididymal tuberculosis on ultrasonography is not distinct from that of bacterial epididymo-orchitis[6]. The most notable ultrasound findings of tuberculous epididymitis are an enlarged epididymis, predominantly in the tail portion, and marked heterogeneity of the echo texture of the involved epididymis [7]. Fine needle aspiration of the epididymis can be useful to distinguish epididymal tuberculosis from bacterial epididymo-orchitis; however, because of the risk of tumor spillage, fine needle aspiration should be avoided if a neoplasm is suspected [8]. A definite diagnosis depends upon positive culture, Ziehl-Neelsen staining and FNAC/ histological examination of the suspected tissue.

The treatment of tuberculous epididymitis consists of epididymectomy in patients with chronic forms and constitutes a diagnostic confirmation procedure [9]. Anti-tubercular treatment consists of combination of four drugs, i.e. rifampicin, INH, ethambutol and pyrazinamide. The duration of treatment is now reduced to 6 to 9 months if the primary drug resistance is ruled out [1, 10].

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Reports of randomized clinical trials should be based on the CONSORT Statement (http://www.consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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