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Editorial

Biofilms and Human Diseases: A Clinician's Nightmare

Ranjana Hawaldar

Author Affiliation
Editor-in-chief, Journal of Microbiology and related research, Sampurna Sodani Diagnostic Clinic, LG-1, Morya Centre, Race Course Road, Indore, Madhya Pradesh.

Reprint Request
Ranjana Hawaldar, Editor-in-chief, Journal of Microbiology and related research, Sampurna Sodani Diagnostic Clinic, LG-1, Morya Centre, Race Course Road, Indore, Madhya Pradesh.
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Microbes have been characterized as planktonic, free-floating single cells. As they grow in culture media, their morphology and physiology properties have been described in detail. But, how they survive in the environment was given very little thought till the concept of biofilms came into existence. The concept of biofilms has opened up a new horizon in the study of microorganisms.

What is Biofilm?

According to the IUPAC definition- Biofilm is an aggregate of microorganisms in which cells that are frequently embedded within a self produced matrix of extracellular polymeric substance (EPS) adhere to each other and / or to a surface [1].

In natural environment, microbes are commonly attached to surfaces as biofilms.

Biofilms are ubiquitous and can be present on both biotic and abiotic surfaces. They are usually found on solid surfaces submerged in /or exposed to aqueous solution. Nearly every species of microorganisms e.g., bacteria, fungi, algae and protozoa adhere to each other and to surfaces by different mechanisms. It has been estimated that nearly 90% of bacteria live in biofilms, whether single species or multiple species of bacteria, fungi, protozoa etc.

Mixed species biofilms are predominantly found in environment while single species biofilms are seen in variety of infections and on medical implant devices [2]. Research has been focused on this aspect in recent years.

It has been estimated that nearly 65% of nosocomial infections in humans are due to biofilms. Due to intrinsic resistance of these micro organisms which form biofilm, they are resistant to most of the antibiotics and diseases caused by them are very difficult to treat.

In favorable conditions, all bacteria are capable of forming a biofilm. Most infections in humans are caused by normal microbial flora of humans which form biofilms at sites where they exist as harmless commensals. In these conditions, biofilms play a protective role for the host. For example, biofilms formed in vagina prevent colonization by exogenous pathogens- a phenomenon called 'colonization resistance' and is healthy for the vagina [3]. However, due to certain exogenous and endogneous factors, this composition gets disturbed and can produce a pathogenic biofilm. Staphylococci, which are normal flora of skin frequently form biofilms on medical devices such as I / V catheters and prosthesis [4, 5, 6]. Similarly, Pseudomonas aeruginosa can cause infections in immune compromised patients [7].

How do Biofilms Develop?

There are five stages in the development of biofilms.

1. Reversible attachment
2. Irreversible attachment
3. Early development
4. Maturation
5. Detachment dispersal of cells

When a microbial cell is <1nm close to a surface, then attachment depends upon the attractive and repulsive forces between the two surfaces [8].

In the second stage, there is a molecular binding between specific microbial adhesion and the surface [9].

Several factors control the formation of biofilm which include:

- ❖ Recognition of attachment sites on a surface,
- ❖ Nutritional cues,
- ❖ Change of pH and temperature,
- ❖ Exposure to antibiotics, chemical biocides and host defense mechanisms.

During colonization, microbial cells communicate by quorum sensing.

Biofilms are formed by many bacteria of medical importance such as Staphylococcus epidermidis, Staphylococcus aureus. Enterococci, Streptococcus mutans, Pseudomonas aeruginosa, E.coli, Neisseria gonorrheae, Vibrio cholerae and fungi such as Candida albicans [10].

Biofilms are a cause of great concern for clinicians and have received much attention in recent years because they are responsible for resistant and persistent chronic infections. They are very difficult to eradicate by routine antibiotics.

Infectious processes caused due to biofilms include bacterial vaginosis, urinary tract infections, catheter related infections, middle ear infections, dental plaque, gingivitis, infection in contact lenses, endocarditis, cystic fibrosis, prosthesis and heart valves [11, 12, 13].

Why are biofilms related infections resistant to many antibiotics and difficult to treat?

Several factors play a role in the resistance mechanisms-

1. Bacteria forming a biofilm are less susceptible to host defense system so the infection can persist for a longer time. This is due to antiphagocytic properties of the biofilm matrix [14, 15].
2. When a bacteria comes in contact with a surface sticky polymers are produced catalyzed by bacterial enzymes which result in colonization and protection. Antibiotics cannot penetrate deeper into the biofilm and can act only on the outer surface. This causes release of large amount of pro inflammatory enzymes and cytokines by the polymorphs which cause tissue damage and chronic inflammation.
3. Biofilms have an innate resistance to antibiotics. The reasons are not very clear but most probably the antibiotics fail to penetrate deep into the biofilms.

   Another plausible reason might be that many antibiotics e.g., Penicillin act only on actively growing cells. Cells that are dormant can re-establish as biofilms once the antibiotic is no longer present in the host body.
4. Biofilms increase the opportunity for gene transfer between the bacteria and among the bacteria. The resistant bacteria transfer the gene for resistance mechanism to neighbouring susceptible bacteria. Similarly a non pathogenic commensal organism can become highly pathogenic by this gene transfer [16].

However, not all bacteria can form biofilms. Some non motile bacteria cannot recognize the surface as easily as motile bacteria, so they cannot form an effective biofilm [14].

Conclusion

Bacteria have been known to cause variety of infections in humans and scientists have been struggling to find out mechanisms by which bacteria invade the host defense mechanism to cause disease. In recent years, drug resistant microbes have been on the rise and the role of biofilms has emerged as a newer concept in causing chronic disease resistant to most of the commonly used antibiotics. Many antibiotics in use are increasingly becoming obsolete as drug resistant bacteria are on the rise. Clinicians are facing newer challenges in treating the patients as the drug options are becoming limited. Furthermore, there has been increasing evidence to support the formation of biofilms in chronic human diseases which are resistant to common antibiotics.

With newer and more sensitive technologies, scientists will be able to gain more insight and better understanding of the biofilm biology which may in the long run help combat bacterial infections which are a clinician's nightmare in the present conditions.

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Original Article

Molecular Characterization of Segment 6 of Indian Isolate of Bluetongue Virus 2

Minakshi Prasad*, Koushlesh Ranjan, Ravinder Singh*, Sandip Khurana***, Gaya Prasad******

Author Affiliation
*Department of Animal Biotechnology, LLR University of Veterinary and Animal Sciences, Hisar, Haryana, 125004. **Department of Veterinary Physiology and Biochemistry, SVP University of Agriculture and Technology, Meerut, Uttar Pradesh, 250110. ***NRCE, Sirsa road, Hisar 125001. ****Indian Council of Agricultural Research, New Delhi, India.

Reprint Request
Minakshi Prasad, Professor and Head, Department of Animal Biotechnology, LLR University of Veterinary and Animal Sciences, Hisar, Haryana, 125004.
E-mail: minakshi.abt@gmail.com

Abstract

Bluetongue (BT) is a disease of domestic and wild ruminant caused by bluetongue virus (BTV) of genus *Orbivirus* and family *Reoviridae*. BTV is non-enveloped icosahedral virus having 10 segmented dsRNA genome. The VP5 protein encoded by segment 6 along with VP2 protein gives serotype specificity to the virus. The BTV serotype 2 (isolate M11) was isolated from an outbreak in sheep from Andhra Pradesh state. The virus sample was grown in BHK21 cell culture. The RNA-PAGE and NS1 gene based RT-PCR confirmed the sample as BTV. The viral cDNA was allowed for segment 6 (vp5 gene) specific RT-PCR using primers for all the serotypes. The BTV2 specific vp5 gene PCR amplicon was cloned and sequenced. Nucleotide sequence analysis revealed that Indian BTV2 serotype showed more than 99 / 97% nucleotide / amino acid sequence identity with western origin viruses from India, USA and South Africa. The study further indicated that western BTV2 in India may be originated from vaccine strains of BTV2 from South Africa.

Keywords: Bluetongue Virus 2; RT-PCR; Topotype; VP5 Gene.

Introduction

Bluetongue (BT) is an infectious, non-contagious, economically important, insect vector borne viral disease of domestic and wild ruminants (MacLachlan, 1994). It is caused by bluetongue virus (BTV), of genus *Orbivirus* of family *Reoviridae*. BT causes severe economic loss to small ruminant industry which is mainly due to its high morbidity, mortality, still birth, foetal abnormality, abortion, wool break, weight loss, reduced milk and meat yield. BT is listed under category of multiple species diseases by Office International des Epizooties, Paris (OIE, 2013). The clinical sign of BT is characterized by fever, swelling of lips and tongue, coronitis and lameness. The more severe forms of the disease are frequently seen in sheep and in white-tailed deer (Darpel *et al.,* 2007; Howerth *et al.,* 1988). Buffalo, cattle and goats act as silent reservoirs host, remain viraemic for several months and may transmit BT to other susceptible host (Maclachlan *et al*., 2009).

Due to rapid evolutionary changes in genome through reassortment, BTVs are consistently evolving new serotypes globally. Twenty-four distinct BTV serotypes have been identified worldwide (Mertens *et al*., 2004). Later on, two more serotypes BTV25 and BTV26 from Switzerland (Hofmann *et al.,* 2008) and Kuwait (Maan et al., 2011) respectively have been isolated. Recently, a novel virus from France has been characterized as BTV serotype 27 (Jenckel et al., 2015). The segmented nature of BTV genome favours the reassortment of genome segments especially when *Culicoides* vector or mammalian host is simultaneously infected by two or more different BTV

serotypes. This may led to evolution of new isolates or serotypes of BTV (Batten et al., 2008). The monsoon season in India is favourable to various insect vectors (including *Culicoides*) and infectious diseases (like BT) transmitted by them. Thus India became endemic for BT and a large number of BTV serotypes (total 22 BTV serotypes) have been reported from India. Based on nucleotide sequence analysis most of BTV isolates can be broadly divided into two major groups 'eastern' or 'western' topotypes, and into a number of geographic subgroups (Balasuriya *et al.,* 2008).

BTV is a small icosahedral virus with a capsid diameter of 70 nm. It has ten-segmented, double stranded RNA (dsRNA) genome, each of which codes for at least one viral protein. The genome segments code seven structural proteins (VP1 to VP7) to form virus particle. In addition to structural proteins, there are four non-structural proteins NS1, NS2, NS3/ NS3a and NS4 expressed in virus infected host cells (Mertens *et al.,* 1989; Belhouchet et al., 2011). The inner capsid of BTV is composed of two major (VP3 and VP7) and three minor proteins (VP1, VP4 and VP6) proteins (Roy, 1989). The outer capsid is composed of viral proteins, VP2 and VP5, which are serotype specific for individual BTV serotype (Ghiasi, 1987).

VP5 protein assists in virus neutralization along with major VP2 protein as it enhances the neutralization activity of VP2 protein (Roy *et al.,* 1990). The combination of VP2 and VP5 proteins can induce higher neutralizing antibody titre in comparison to VP2 protein alone (Huismans *et al.,* 1983). In addition VP5 can also play major role in membrane fusion and membrane permeabilization activity, facilitating virus entry into the host cells (Forzan *et al.,* 2004). The segment 6 (VP5 gene) of BTVs also showed genetic diversity between serotypes as well as within serotype (Singh *et al.,* 2004). In present paper, VP5 gene based genetic variation and phylogenetic study of Indian BTV2 isolate with global isolates has been reported.

Materials and Methods

Sample Origin

The BTV serotype 2 was originally obtained from Andhra Pradesh state under All India Network Program on Bluetongue in 2004. It was assigned as isolate M11. The Blood sample of Nellore breed of sheep was collected at height of temperature (40.5ºC). The sample was washed with sterile PBS of pH 7.4 and allowed for ultra-sonication and intravenous inoculation to 9-11 day old chicken embryo. The embryos showing characteristic embryopathic effect were harvested within 7 days and embryonic tissues were inoculated to day old confluent monolayer of BHK21 cell line. After showing characteristic cytopathic effect (CPE), it was passaged for 10 passages in BHK21 cell culture. After appearance of 75% CPE in infected cell culture, the virus was harvested along with BHK21 cells and cellular material was pelleted at 5000Xg using centrifuge machine (Remi, India). The viral dsRNA was extracted from pelleted cell culture material using Trizol reagent (Sigma, USA) as per manufacturer's instruction. The extracted viral nucleic acid was screened by RNA-PAGE followed by silver staining to visualize the 10 dsRNA segments to confirm the sample as BTV.

RT-PCR and PCR

The cDNA was synthesized using viral genomic dsRNA of M11 isolate as template, random decamer primers (Ambion, USA) and moloney murine leukemia virus-reverse transcriptase (Mo-MuLV-RT) enzyme (Sibzyme, Russia) in thermal cycler (Biorad i-Cycler, USA) as per manufacturer's instruction. The cDNA was subjected to group specific NS1 gene based PCR to confirm the sample as BTV using forward primer (11-31 nucleotide): 5'GTTCTCTAGTTGG CAACCACC3' and reverse primer (284-265 nucleotide): 5' AAGC CAGACTGTTTCCCGA 3' to generate an amplicon of 274bp size (Prasad et al., 1999). The cDNA was subsequently subjected to amplification of VP5 gene using published primer pairs specific to BTV2 (Ranjan et al., 2012) along with primers specific to all other BTV serotypes. The VP5 gene specific primers of BTV serotype 2, Forward (755-774 nucleotide) - 5'ACAGCCGTCGCAACGGG AAG3' and Reverse (1589-1570 nucleotide) - 5'AGAGGGGCACGTCCAACCGA 3' produces the PCR amplicon of 835 bp (Ranjan et al, 2012). The amplification reaction for BTV2 specific primer was carried out in 20 µl reaction mixture containing 2 µl cDNA, 3% DMSO, 20 µM of each primer along with 0.4 µl of 10mM dNTPs mix, 4 µl 5X HF buffer and 0.4 U (2U / µl) phusion high- fidelity DNA polymerase (Finnzymes, Finland) in thermal cycler (Biorad iCycler, USA). The amplification programme consisted of initial denaturation for 3 minute at 98°C, followed by 35 cycles for 15 second denaturation at 98°C, 20 second primer annealing at 57°C and 30 second primer extension at 72°C. The final primer extension was carried out at 72°C for 10 minute. Similarly cDNA was allowed for PCR using VP5 gene specific primers of all other serotypes. The amplified

PCR products were subjected to agarose gel electrophoresis in 1% agarose gel (Sigma, USA) followed by ethidium bromide staining and UV visualization under transilluminator (Biovis, USA).

Cloning and Sequencing of PCR Product

The PCR products were purified using commercially available QIA quick gel extraction kit (Qiagen, USA) to remove primer dimers and other PCR ingredients. The purified PCR products were cloned using commercially available cloning kit (Fermentas, USA) as per the manufacturer's instruction. The Pjet 1.2 plasmid was used as cloning vector and JM107 *E. coli* cell line as host system. The positive clones were selected by colony touch PCR using VP5 gene specific primer pairs. The positive clones were further grown in culture media and plasmids were extracted using commercially available kit Quiaprep (Quiagen, USA) as per the manufacturer's instruction. The plasmids from positive clones were allowed for nucleic acid sequencing using Genetic Analyser ABI PRESM ™ 3130 XL machine in our laboratory.

Nucleotide Sequence Analysis

The vector contamination sequence from nucleotide sequence obtained was removed using online software Vecscreen (http: //www. ncbi. nlm.nih. gov /tools /vecscreen/). The VP5 gene sequence of M11 isolate was analysed using online available software program BLASTN+ 2.2.31 (http: //blast.ncbi.nlm.nih.gov/) (Zhang *et al.,* 2000). Bioedit v7.2.1 software (Hall, 1999) was used for multiple sequence alignment and calculation of percent identity of nucleotide as well as deduced amino acid sequence of M11 isolate with other global isolates of BTV2. The phylogenetic analysis of M11 isolate with other isolates of BTV2 from different parts of the world was done using neighbour joining method with p-distance parameter and 1000 bootstrap value in Mega 5 software (Tamura *et al.,* 2011).

Result and Discussion

BTV 2 is one of the common BTV serotypes reported from Andhra Pradesh state of India. The segment 6 sequence based study showed significant genetic diversity (3-43%) among BTV isolates of all the 24 serotypes of BTV across the world (Singh *et al.,* 2004). The present study was carried out to investigate the vp5 gene based molecular variation of Indian isolate of BTV2 and its comparison with other BTV2 isolates from India and other countries. The dsRNA of cell culture grown BTV isolate (M11) was analysed using 8% RNA-PAGE. The RNA-PAGE analysis showed characteristic 3:3:3:1 migration pattern which is specific for BTV (Data not shown) (Minakshi et al., 2011). Furthermore, the group specific NS1 gene based PCR of viral cDNA revealed BTV specific amplicon of 274 bp in agarose gel electrophoresis (Figure 1). Thus, RNA-PAGE and NS1 gene specific PCR confirmed the sample as BTV. The VP5 gene based PCR of M11 isolate showed an amplicon of 835 bp with BTV2 specific primers as evidenced in 1% agarose gel electrophoresis (Figure 2). However, the cDNA sample did not show any amplification with primers specific to other serotypes. Subsequently, the BTV2 segment 6 (VP5 gene) specific PCR amplicon was cloned using PJet 1.2 cloning vector and sequenced to get complete nucleotide sequence of amplicon. After removal of terminal end vector sequences the VP5 gene nucleotide sequences showed identity with several isolates of BTV2 of GenBank database only. The nucleotide sequence of M11 isolate was deposited to GenBank database with accession number JF815523. The percent nucleotides (nt) as well as deduced amino acid (aa) sequence identity of isolate M11 with other BTV2 isolates from different regions of the world was calculated using Bioedit v7.2.1 (Table 1). The M11 isolate showed >86/95% nt/aa identity with western topotype isolates of BTV2 from different regions of the world. However, it showed maximum 99/97% nt/aa identity with several western BTV2 isolates from India (sheep/08/Ind/ABT/Hisar, IND2003/02, BTV-2/IND2003/01, IND2003/01, IND2003/03 and BTV-2/IND2003/03), USA (OnaA) and South Africa (557, RSArrrr/02 and RSAvvvv/02). Moreover, it showed only 75.6-78.4/91.0-91.7% nt/aa identity with eastern topotype isolates of BTV2 from India (IND1982/01 and BTV-2/IND1993/01), Japan (MZ-1/C/07) and Australia (Cooktown). It confirmed the western origin of segment 6 of M11 isolate.

Phylogenetic analysis of segment 6 classifies BTV2 into separate eastern and western topotypes (Fig. 3). The M11 isolate was placed within western topotype and it formed a separate close cluster with BTV2 from India (IND2003/02, sheep/08/Ind/ABT/Hisar, BTV-2/IND2003/01, IND2003/01, IND2003/03 and BTV-2/IND2003/03), USA (OnaA) and South Africa (557, RSArrrr/02 and RSAvvvv/02). However, it was slightly distant from other western topotype viruses from France, Italy, Tunisia, Nigeria, Sudan and USA, which formed separate cluster (Fig. 3). The eastern topotype was consists of BTV2 form India (IND1982/01 and BTV-2/IND1993/01), Australia (Cooktown) and Japan (MZ-1/C/07). The segment 6 (VP5 gene)

based molecular characterization and phylogenetic analysis of Indian isolate of BTV1, 2, 9 and 16 have been reported earlier (Manjunath *et al.,* 2010; Ranjan et al., 2013; Ranjan et al., 2014a; 2014b). The present study indicates that BTV2 serotype having both eastern as well western origin of segment 6 (VP5 gene) is circulating in India. Many of the western origin of BTV2 isolates in India including M11 isolate showed >99/97% nt/aa identities with South African live attenuated vaccine strain (RSAvvvv/02). Therefore, western origin of BTV2 might have entered to India either through import of live vaccinated animals or live vaccine itself. In South African BT control programme multivalent live attenuated vaccine containing BTV 2, 3, 4, 8-11, 16 had been used. Moreover, these vaccines were also imported by many other countries including Italy, Bulgaria, France, Spain, Portugal, Israel, eastern Mediterranean Islands (Savini et al., 2008; Coetzee et al., 2012). The several sheep breeds were imported to India during late 1970s and 1980s from these countries (Prasad et al., 2009; Jain et al., 1986). Furthermore, it was well established that live vaccines may cause significant levels of post vaccination viraemia leading to virus transmission by *Culicoides* vector during blood feeding. It may lead to either revert back of vaccine strain to virulent strain or reassortment of vaccine strain with other locally prevalent BTV isolate (Veronesi et al., 2010; Batten et al., 2008). Thus, new BTV isolate or serotype originated in an area. Moreover, the BTV2 was isolated from southern India especially from Andhra Pradesh and Tamil Nadu during 2003-04. All the western isolates of BTV2 were found to be sharing more than 99/97% nt/aa identity. It also indicates their common origin. Since, south India particularly Andhra Pradesh and Tamilnadu is endemic for a major species of *Culicoides* i.e. *Culicoides oxystoma* (Minakshi, 2010). Therefore, BTV2 serotype after entering to Indian subcontinent may be migrated either from Tamil Nadu to Andhra Pradesh or vice versa through migrating sheep population or vector.

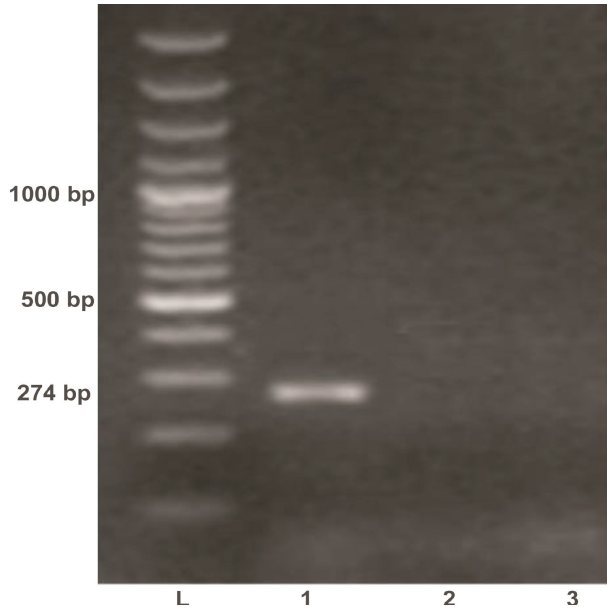

Fig. 1: Agarose gel electrophoresis of RT-PCR product of ns1 gene of M11 isolate. Lane L: Ladder 100bp, 1: M11, 2: BHK21 cell control, 3: NFW control.

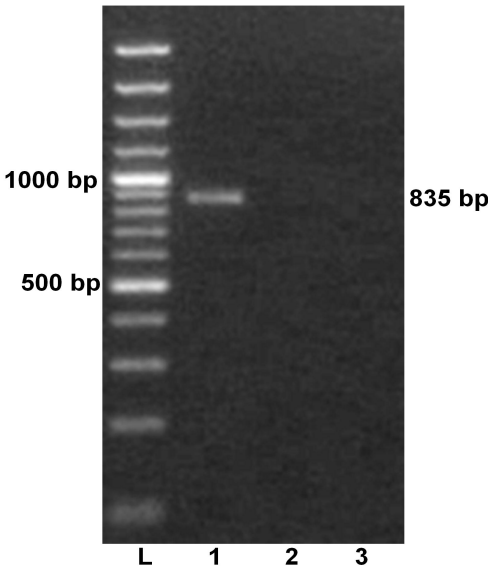

Fig. 2: Agarose gel electrophoresis of RT-PCR product of vp5 gene of M11 isolate. Lane L: Ladder 100bp, 1: M11, 2: BHK21 cell control, 3: NFW control

Table 1: Percent nucleotide and deduced amino acid sequence identity of segment 6 of bluetongue virus serotype 2

S.n.	BTV2 vp5 gene sequences	BTV2.M11.India.JF815523	
		Nucleotide	Amino acid
1	BTV2.M11.India.JF815523	100	100
2	BTV2.sheep/08/Ind/ABT/Hisar.India.JQ904068	99.1	97.4
3	BTV2.IND2003/02.India.AJ783905	99.1	97.8
4	BTV2.557.South Africa.AY855276	99.2	97.8
5	BTV2.OnaA.USA.AY855277	99.2	97.8
6	BTV2.BTV-2/IND2003/01.India.KP696597	99.2	98.2
7	BTV2.IND2003/01.India.AJ783904	99.2	98.2
8	BTV2.IND2003/03.India.AJ783906	99.4	98.2
9	BTV2.BTV-2/IND2003/03.India.KP696607	99.4	98.2

10	BTV2.RSArrrr/02.South Africa.AJ586696	99.2	97.8
11	BTV2.RSAvvvv/02.South Africa.AJ586665	99.0	97.1
12	BTV2.France.AY129083	87.4	96.7
13	BTV2.BTV-2IT2000.Italy.KM053273	87.4	97.1
14	BTV2.FRA2001/03.France.AJ586674	87.4	96.7
15	BTV2.SAD2001/01.Italy.AJ586672	87.4	96.7
16	BTV2.ITL2002/07.Italy.AJ586671	87.3	96.4
17	BTV2.TUN2000/01.Tunisia.AJ586668	87.5	97.1
18	BTV2.NIG1982/02.Nigeria.AJ586667	87.1	96.7
19	BTV2.SUD1985/01.Sudan.AJ586666	87.6	97.1
20	BTV2.FL99 13406-2.USA.AY855279	85.7	96.0
21	BTV2.OnaB.USA.AY855278	86.2	95.6
22	BTV2.MZ-1/C/07.Japan.AB686238	78.4	91.0
23	BTV2.Cooktown.Australia.JQ240326	79.5	91.0
24	BTV2.IND1982/01.india.AJ586675	78.2	91.7
25	BTV2.BTV-2/IND1993/01.India.KP696587	75.6	91.0

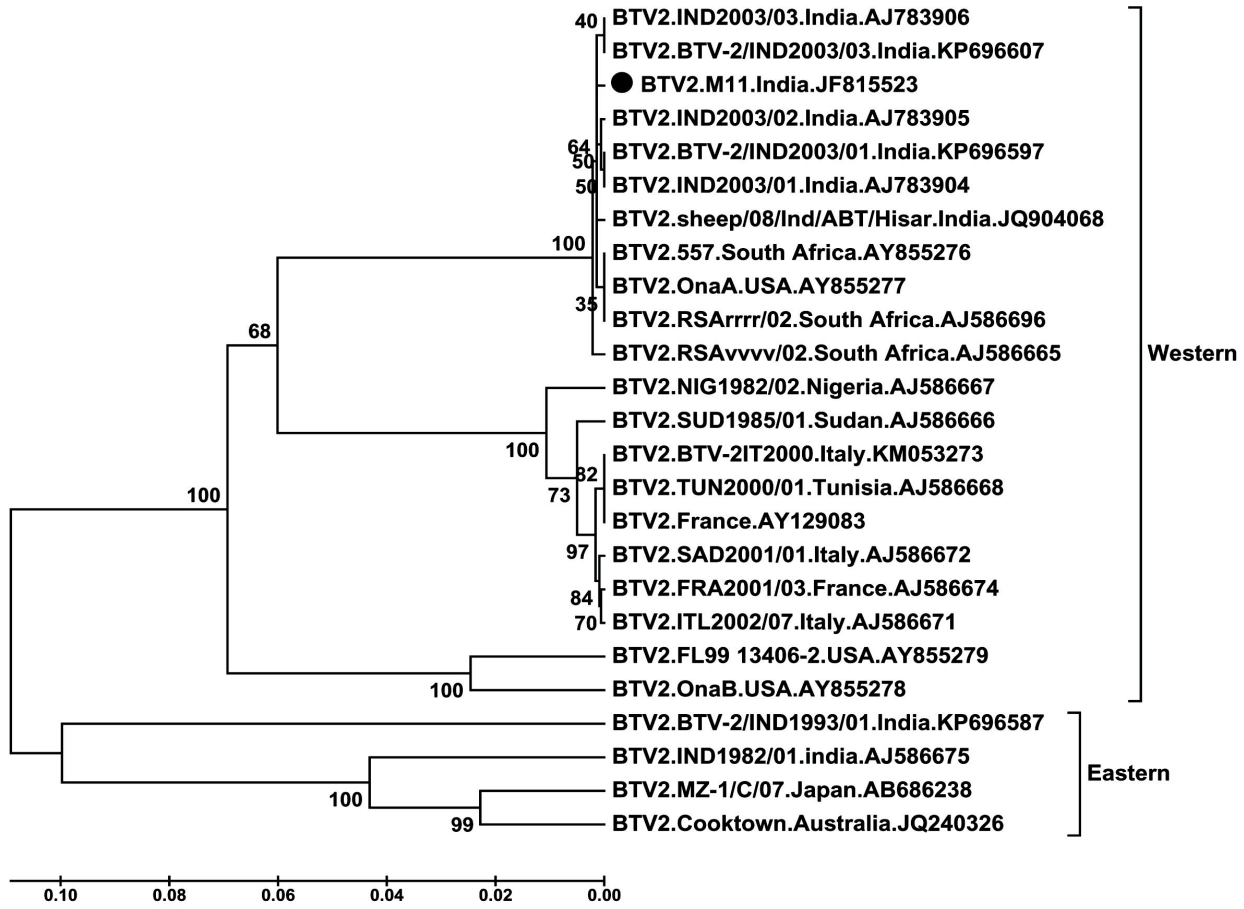

Fig. 3: The vp5 gene based phylogenetic tree of M11 isolate along with other BTV2 isolates from around the world. Tree was constructed using neighbour joining method in Mega5 software programme with default parameters and bootstrap value as 1000 replicate (Tamura *et al.,* 2011). ● = Isolate selected in this study

Conclusion

Based on percent nucleotide and deduced amino acid sequence identity of segment 6 of Indian isolate of BTV2 and phylogenetic analysis with global isolates, it can be concluded that M11 isolate is of western origin and more closely related to South African vaccine viruses. Moreover, the segment 6 sequence analyses also revealed little genetic divergence among western topotype of Indian BTV2 isolates. It indicates their common origin from South African Vaccine virus.

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Original Article

Study of Opportunistic Infection in Relation with CD4 Cell Count and HIV Prevalence

Charushila S. Halgarkar*, S. L. Nilekar, R. S. Ovhal****

Author Affiliation
*****Assistant Professor
******Associate Professor,
Department of Microbiology,
S.R.T.R. Medical College,
Ambajogai - 431517,
Maharashtra.

Reprint Request
Charushila S. Halgarkar,
Asst. Professor, Department
of Microbiology, S.R.T.R.
Medical College, Ambajogai –
431517, Maharashtra.
E-mail:
dr.charushilahalgarkar@gmail.com

Abstract

Background: In India, an opportunistic infection with HIV and associated complications accounts for considerable proportion of mortality. There exists definite CD4 cell count correlation with opportunistic infection in HIV patients. *Objectives:* To document the prevalence of HIV with correlation of different opportunistic infection with CD4 cell count. *Material and Method:* A total of 174 HIV positive patients either hospitalized or ART POD were studied for finding the spectrum of opportunistic infection and for HIV prevalence. Various samples were collected as per symptoms and clinical presentation. *Result:* Among opportunistic infection, most common were bacterial infection with 72.5%, followed by fungal infection 42.1% and parasitic infection with 25.8%. TB in 21 patients and candidiasis in 5 patients were found in CD4 cell count <500 cell / µl followed by chronic diarrhoea with CD4 cell count <200 cell / µl. *Conclusion:* Prevalence of HIV infection in persons attending ICTC is 11.3%. TB is the most common opportunistic infection followed by candidiasis and diarrhoea.

Keywords: CD4 Cell Counts; OIs (Opportunistic Infections); HIV Prevalence.

Introduction

AIDS is a disease which came in light only in 1981. Presently HIV accounts for highest number of death attributable by single infective agent. Opportunistic infection associate complication account for considerable proportion of mortality. Opportunistic infections also cause substantial mortality and hospitalization necessitates toxic and expensive therapies and shortens the survival of people with HIV infection [2, 4].

Early diagnosis of opportunistic infection and prompt treatment definitely contributes to increased life expectancy among infected patients delaying the progression of AIDS [1].

We, therefore, conducted this HIV prospective observational study to document the HIV prevalence pattern and correlation of different opportunistic infections with CD4 cell count.

Material and Method

A prospective observational cohort study was conducted for prevalence of HIV, considering all persons attending integrated counseling and testing centres (ICTC). Blood samples were collected and tested with patients consent.

After they were identified as HIV positive, post test counseling was given and they were referred to ART centre in our hospital. For finding the spectrum of opportunistic infection, a total of 174 HIV positive patients either hospitalized or attending ART OPD were studied during a period of 1 year from June 2012-June 2013.

CD4/CD3 Enumeration

The CD4 / CD3 enumeration was done using the single BD FACS count machine Becton Dickinson and company, San Jose, and the United States of America, strictly follow the manufacturer's instructions.

Table 1: Age and Sex wise distribution of symptomatic patients studied for opportunistic infections

Age (Yrs)	Male	Female	No. of patients	Percentage
18 -28	22	7	29	17.4 %
29-38	71	23	94	53.3 %
39-48	31	5	36	21 %
49 -58	10	3	13	7.3 %
59 and above	2	0	2	1 %
Total	136	38	174	100 %

Depending on the patients clinical features various specimens were collected which included a stool, sputum and oral swabs all three sputum samples were used to make separate smears and stained by a Ziehl - Neelson method.

Stool specimens were collected and examined microscopically using saline wet mounts. Lugol's iodine was used for the detection of ova, larva, trophozoites and cysts of intestinal parasites. Smears were examined by Modified Acid Fast Staining for Cryptosporidium parvum, Isospora belli and other. Also bacterialogical culture of stool was done for identifying bacterial infections.

Candidiasis were diagnosed by taking oral swab specimens and were cultured on Sabourad's Dextrose agar and suspected colony was identified by Germ tube test. Cryptococcal meningitis was diagnosed by using India ink preparation of CSF and by culture on SDA.

Observations

In this study out of 174 patients of opportunistic infections maximum 53.3% were in the age group of 29-35 years, followed by 21 % in the age group of 39-48 years. 17.4% were in the age group of 18-28 years while 7.3 % were in 49 - 58 years age group. Only 1% of patients were in 59 and above age group.

Table 2: Distribution of opportunistic infections

Infection	No. of patients	Percentage
Bacterial	129	72.5 %
Fungal	75	42.1 %
Parasitic	46	25.8 %

It was found that 77% were male and 23% were females, with male to female ratio is 3.09:1.

Among different opportunistic infections, bacterial infections were seen in 72.5% patients followed by fungal in 42.1% and parasitic infections in 25.8% patients.

In this study total 250 events of opportunistic infections were found comprising of bacterial, fungal and parasitic infections.

Opportunistic Infections Associated with CD4 Cell Counts less than 50 cells/µl

Among bacterial infections 3 patients of TB were found in this age group. Among the fungal, Candida species found in 4 patients followed by Cryptococcus neoformans in 2 patients. Among parasitic infections only *cryptosporidium parvum* were isolated in 3 patients.

Opportunistic Infections Associated with CD4 Cell Counts between 51- 100 cells/µl

TB were found in 11 patients followed by S. aureus in 2 and *Klebsiella pneumoniae* in 1 patient. *candida sp.* Found in 6 patients followed by Cryptococcus neoformans in 3 among fungal infections. In parasitic infections *cryptosporidium parvum* was isolated in 5 patients followed by *Strongyloides stercoralis* and *Giardia lamblia* in 1 patient each.

Opportunistic Infections Associated with CD4 Cell Counts between 101-150 cells/µl

Here TB was found in 18 patients followed by klebsiella pneumonia in 2 patients, *Staph.* aureus, strep. *Pneumoniae* and *Salmonella typhi* were found in 1 patient each. *Cryptosporidium parvum* was isolated in 10 patients, *H.nana* in 3 patients. *Isospora belli* and *Entamoeba histolytica* were found in 2 patients each. In fungal, Candida species were found in 17 patients.

Oppotunistic Infections Associated with CD4 Cell

Table 3: Correlation of OIs with CD4 cell counts

Type of Etiology	Opportunistic pathogen		CD4 cell count I (<50)	II (51 – 200) IIa (50 - 100)	IIb (101-150	IIc (151-200)	III (201-500)	Total
Bacterial	*Mycobacterium tuberculosis*	Pulmonary	2	7	11	30	12	62
		Ext. pulmonary	1	4	7	22	9	43
	Klebsiella pneumoniae		-	1	2	6	2	11
	Staphylococcus aureus		-	2	1	3	-	6
	Streptococcus pneumoniae		-	-	1	2	-	3
	Escherichia coli		-	-	-	2	-	2
	Salmonella typhi		-	-	1	1	-	2
Fungal	*Candida spp.*		4	6	17	35	5	67
	Cryptococcus neoformans		2	3	-	-	-	5
	Trichophyton rubrum		-	-	1	1	1	3
Parasitic	*Cryptosporidium parvum*		3	5	10	14	-	32
	Isospora belli		-	-	2	2	-	4
	Strongyloides stercoralis		-	1	1	1	-	3
	Hymenolepsis nana		-	-	3	-	-	3
	Entamoeba histolytica		-	-	2	-	-	2
	Giardia lamblia		-	1	1	-	-	2
Total			12	30	60	119	29	250

Counts between 151- 200 cells/µl

Here TB found in 52 patients, followed by klebsiella pneumoniae in 6 patients. Candida found in 35 patients and *Trichophyton rubrum* in 1 patient. In parasitic infections *Cryptosporidium parvum* was isolated in 14 patients. In present study only TB and Candidacies were found in the CD4 cell count range of 201-500. In the CD4 cell count range of 51-200, along with TB and Candidacies, the most common infections found were cryptosporidiosis and other parasitic diarrhoea. *Cryptococcal meningitis* was found in CD4 cell count of less than 50.

Discussion

Out of 174 patients under study, 250 isolates of OIs were seen in present study singly / in mixed form. Among bacterial infections TB was found to be most common bacterial infections which were seen in 59% of patients. After TB, next common bacterial infections found were bacterial pneumonia in 11.8% of patients. Fungal infections were second common infections found in 42.1% of patients after bacterial infections. Among those candidacies was found to be the most common [2, 6] that is 37.6% in present study. After bacterial and fungal infections next common group of infection was found to be parasitic infections in 25.8% patients. Among parasitic infections cryptosporidium parvum was the commonest organism causing diarrhoea [5].

For correlation of OIs with CD4 cell count, we divided the study cases into three groups based on CD4 cell counts (cells / mm3) that is <50, 51-200, and 201- 500 cells / mm3. In this study we found out of 250 isolates of OIs only TB and candidacies were found in range of 201-500 cell / mm3 [3].

In this study, CD4 cell count range of 51-200, we found TB, candidacies and parasitic diarrhoea and other bacterial infections. We also found cryptococcal meningitis in patients of CD4 cell count range

50-100 cells / mm3 [8].

When severe immunodeficiency occur that is CD4 cell count less than 50 cell / mm3, almost all OIs become manifest at this terminal stage of AIDS. However we found only TB, candidacies, cryptococcal meningitis, and diarrhoea due to cryptosporidium parvum. This might be because we have got less patients with CD4 cell counts <50 cells / mm3.

Table 4: Correlation of opportunistic infections with CD4 count

CD4 counts (cells / µl)	Opportunistic infections
200 -500	T.B. Candidacies.
51-200	TB, Candidacies, Cryptosporidiosis, other parasitic diarrhoea, bacterial pneumonia.
< 50	Cryptococcal meningitis, cryptosporidiosis, candidacies,

Conclusion

In the present study, prevalence of HIV persons attending ICTC is 11.3%. Male to female ratio was 3.09:1%. Maximum cases were found 20-40 years age group. Among OIs most common were bacterial infections (72.5%), followed by fungal infections (42.1%), and parasitic infections (25.8%).

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Original Article

Study of Aerobic Isolates from Vaginal Swabs in Pregnant Women in a Stand-alone Diagnostic Laboratory of Central Madhya Pradesh

Sodani Sadhna*, Hawaldar Ranjana**

Author Affiliation
*Assistant Professor, Dept. of Microbiology, MGM Medical College, Indore.
**Sampurna Sodani Diagnostic Clinic, Indore

Reprint Request
Ranjana Hawaldar,
Sampurna Sodani Diagnostic Clinic, LG-1, Morya Centre, Race Course Road, Indore, Madhya Pradesh
E-mail: drranjana@sampurnadiagnostics.com

Abstract

Introduction: Colonisation of vagina and cervical epithelium by a variety of micro organisms can cause pelvic infection in pregnant women. The pH and enzymes present in the vaginal secretions determines the type of micro organisms which may cause infection. The knowledge of the commensal bacterial flora of vagina and cervical epithelium is important in pregnancy as many complications may arise due to infection like premature rupture of membranes, puerperal fever, intra or post partum neonatal infections via upper respiratory tract or the umbilical cord Bacterial vaginosis (BV) occurs in about 20 % of the pregnant women and has been associated with adverse pregnancy out comes. The present retrospective study was undertaken with the primary aim of analyzing the common micro organisms causing vaginal infections in pregnant women coming to our microbiology department for vaginal swab cultures. *Materials and Methods:* High vaginal swabs were collected from 267 pregnant women coming to our Microbiology Department with vaginal symptoms from June 2014 to May 2015. Samples were collected with sterile swabs and immediately inoculated on blood and MacConkey agar plates. After 24 hours, Gram's stain of pure colonies was done to indentify the organism and accordingly panel was selected for identification and sensitivity on Vitec II (Biomerieux). *Results:* A total of 159 / 267 (59.5 %) cultures were sterile. 108 / 267 (40.5 %) cultures showed growth of micro organisms out of which E.coli was isolated in 22 (20.3 %), Candida (33.3 %), Staph. aureus (20.3 %), group B Streptococci (11.1 %) and Klebsiella (2.7 %). Candida species was the commonest organism isolated, followed by Staph aureus and E.coli, group B Streptococci and rarely Klebsiella . *Conclusion:* Lactobacilli constitute the major vaginal flora in women during child bearing age and is helpful in maintaining the acidic pH of vagina so that organisms do not invade and cause disease. The natural history of abnormal vaginal flora in pregnancy is still poorly understood but that it can cause perinatal morbidity and mortality is a well known fact. So, for a healthy perinatal outcome of pregnancy, a vaginal swab culture is required in all pregnant women.

Keywords: Lactobacilli; Bacterial Vaginosis; Premature Rupture of Membranes.

Introduction

Colonisation of vagina and cervical epithelium by a variety of micro organisms can cause pelvic infection in pregnant women [1]. The pH and enzymes present in the vaginal secretions determines the type of micro organisms which may cause infection [2]. The knowledge of the commensal bacterial flora of vagina and cervical epithelium is important in pregnancy as many complications may arise due to infection like premature rupture of membranes, puerperal fever, intra or post partum neonatal infections via upper respiratory tract or the umbilical cord [3, 4].

The vaginal flora of healthy asymptomatic women is determined by Lactobacillus which has a protective

role to play in maintaining the natural healthy balance of vaginal flora especially during pregnancy. Lactobacilli are responsible for keeping the pH of the vagina below 4.5 by lactic acid production, which in turn inhibits the growth of non-acid tolerant micro organisms which are potentially pathogenic [5,6]. Lactobacillus species also produce hydrogen peroxide which is toxic to other micro organisms [7].

The normal vaginal bacterial flora is predominantly lactobacilliary type while abnormal flora consists of both aerobic and anaerobic micro organisms such as E.coli, group B streptococci, Enterococci, Candida species etc. Bacterial vaginosis occurs due to a change in the bacterial micro flora of vagina.

During physiological pregnancy, the high estrogen levels induce a better epithelial tropism and also an increased lactobacillary activity due to increased availability of glycogen, as the pregnancy advances, the bacterial flora of vagina changes. When lactobacilli decrease, the incidence of vaginal infection increases causing many adverse perinatal outcomes such as preterm labour, pre mature rupture of membranes, preterm birth and perinatal infection.

Bacterial vaginosis (BV) occurs in about 20 % of the pregnant women and has been associated with adverse pregnancy out comes [8].

The mechanism by which BV causes adverse pregnancy out comes is not yet well understood but may be due to alterations in the host defense mechanisms that can cause ascending intra uterine infection especially in immune compromised patients [9]. On the other hand, women with an exaggerated response to bacterial stimulus release large amount of cytokines at the maternal foetal interface and are at an increased risk for preterm labour if the bacteria gain access to the choriodecidual space. Micro organisms are recognized by Toll-like receptors which initiate release of inflammatory chemokines and cytokines. These trigger prostaglandin production in the amnion, chorion, decidua and myometrium leading to uterine contractions, cervical dilatation, and rupture of membranes, which in turn leads to bacterial entry into the uterine cavity. 40 % of these peripartum febrile illnesses are caused by intra amniotic infections and early onset neonatal sepsis. The incidence increases with decreasing gestational age at delivery [10].

The present retrospective study was undertaken with the primary aim of analyzing the common micro organisms causing vaginal infections in pregnant women coming to our microbiology department for vaginal swab cultures.

Materials and Methods

High vaginal swabs were collected from 267 pregnant women coming to our microbiology department with vaginal symptoms from June 2014 to May 2015. Vaginal swabs were collected from pregnant women whether asymptomatic or with symptoms of abnormal vaginal discharge, itching, burning and lower abdominal pain.

Samples were collected with sterile swabs and immediately inoculated on blood and MacConkey agar plates to prevent drying. Simultaneously a wet smear and Gram's smear was examined for presence of pus cells clue cells, yeast cells, Trichomonas and other bacterial flora.

All plates were incubated at 37 degree Celsius for 24 hours. After 24 hours, Gram's stain of pure colonies was done to indentify the organism and accordingly panel was selected for identification and sensitivity on Vitec II (Biomerieux).

Results

Total of 267 samples were analyzed during June 2014 to May 2015. The patients were divided into age groups of < 20 years (4 / 267, 1.4 %), 20 – 25 (46 / 267, 17.2 %), 26 – 30 (88 / 267, 32.4 %), 31 – 35 (65 / 267, 24.3 %,)36 – 40(29 – 267, 10.8 %), 41 – 45 (13 / 267, 4.86 %) and more than 45 years (22 / 267, 8.2 %). Table 1 shows the demographic data of these patients.

The organisms isolated in these groups are shown in table 2.

In < 20 years age group, no pathogenic micro organisms were isolated.

In 20 – 25 years age group 32 cultures were sterile, 1 each was E.coli, Staph. aureus and group B streptococci. Candida sps was isolated in 6 cases.

In 26 – 30 years age group, 40 cultures were sterile, 7 were E.coli, 18 were Candida sps, 8 were Staph. aureus and 4 were group B Streptococcus.

In 31 – 35 years age group, 40 cultures were sterile, 5 showed growth of E.coli, 6 were Candida sps, 4 were Staph. aureus, 2 each were group B Streptococcus and Klebsiella.

In 41 – 45 years age group, 10 cultures were sterile, 1 each was E.coli, Klebsiella and group B Streptococcus and 2 were Staph. aureus.

In the age group above 45 years, 13 cultures were sterile, 4 showed growth of E.coli, 3 were Candida and 1 each were Staph. aureus and group B Streptococci.

A total of 159/267 (59.5%) cultures were sterile. 108 / 267 (40.5 %) cultures showed growth of micro organisms out of which E.coli was isolated in 22 (20.3 %), Candida (33.3%), Staph. aureus (20.3 %), group B Streptococci (11.1%) and Klebsiella (2.7%). Candida species was the commonest organism isolated, followed by Staph. aureus and E.coli, group B Streptococci and rarely Klebsiella (table 3).

Maximum micro organisms were isolated in 26 – 30 years age group. No pathogenic organisms were isolated in age group below 20 years maybe because there were only 4 patients in this study group. Maximum patients were in 26 – 30 years age (88 / 267) group followed by 31 – 35 years age (65 / 267) group and 36 – 40 years of age (29/267).

The sensitivity pattern of the organisms isolates is shown in table 4.

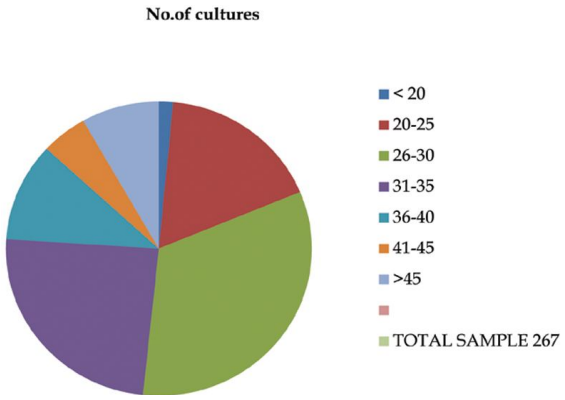
The maximum growth in 26 – 30 years age group followed by 31 – 35 years age group is probably because this is the best child bearing age group and most pregnancies occur in these ages.

Table 1:

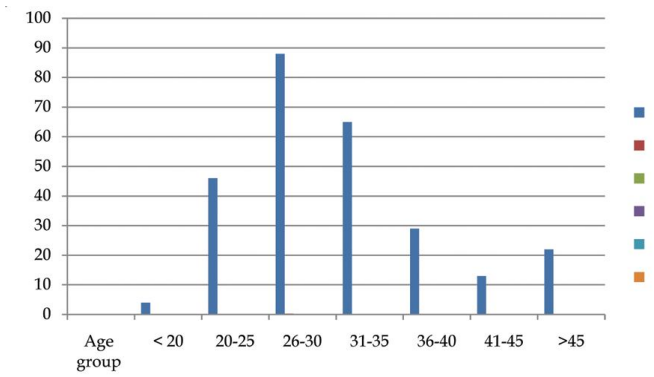
Age group	No. of cultures	Percentage
< 20	4	1.41%
20-25	46	17.20%
26-30	88	32.40%
31-35	65	24.30%
36-40	29	10.80%
41-45	13	4.86%
>45	22	8.20%
Total Sample	267	

Table 2: Isolates in different age groups

	Organisms					
Age group	**Sterile**	**E.coli**	**Candida**	**Staph .aureus**	**Strepto coccus**	**Klebsiella**
< 20	4	0	0	0	0	0
20-25	32	1	6	1	1	0
26-30	60	7	18	8	4	0
31-35	40	5	6	4	2	2
36-40	16	3	3	6	3	0
41-45	10	1	0	0	1	1
>45	13	4	3	1	1	0



Graph 1:



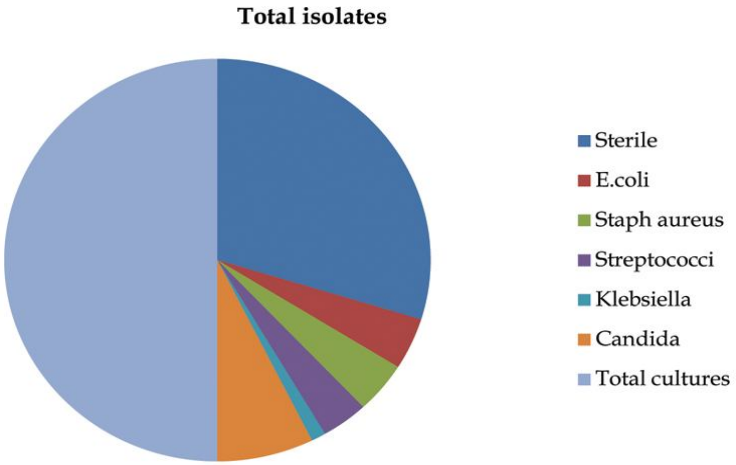
Graph 2:

Table 3:

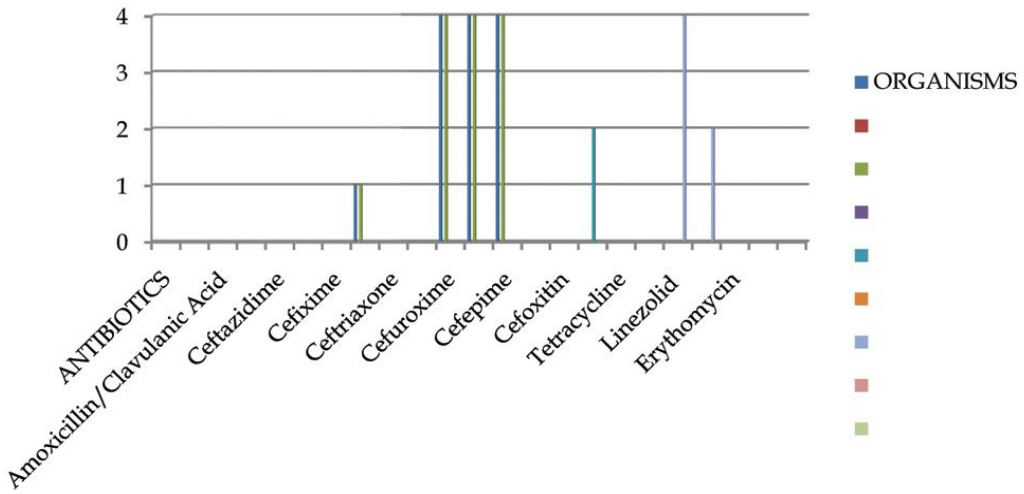
Organisms	Total isolates	Percentage
Sterile	159	59.50%
E.coli	22	20.30%
Staph aureus	22	20.30%
Streptococci	19	11.11%
Klebsiella	6	2.70%
Candida	39	33.30%
Total cultures	**267**	

Table 4: Sensitivity pattern of different isolates

	Organisms							
Antibiotics	**E.coli**		**Klebsiella**		**Staphylococcus**		**Streptococcus**	
Ampicillin	<=2	S	<=2	S	<=8/4	R		
Amoxicillin/Clavulanic Acid	<=2	S	16/8	I	<=4/2	R	<=4/2	
Amikacin	<=2	S	<=16	S	>32	R		
Ceftazidime	>=64	R	>=64	R				
Cefalotin	>=64	R	>=64	R	<=8	R		
Cefixime	>=64	R	>2	R				
Ciprofloxacin	1	S	1	S	>2	R	<=1	
Ceftriaxo ne	<=1	S	<=1	S				
Colistin	<=0.5	S	<=0.5	S				
Cefuroxime	4	S	4	S				
Ertapenem	4	S	4	S				
Cefepime	4	I	4	I				
Fosfomycin	<=16	S	<=16	S				
Cefoxitin	>=64	R	>=64	R	>4	POS		
Vancomycin					2	S		
Tetracycline	>8	R	>8	R	<=4	S	>8	R
Teicoplanin					>8	S	<=8	
Linezolid					<=1	S	4	
Levofloxacin	<=2	S	>4	R	>4	R	2	S
Erythomycin					>4	R		
Clindamycin					<0.25	R*	<=0.25	S



Graph 3:



Graph 4:

Discussion

Bacterial vaginosis is the commonest infection in women of reproductive age group and is an important cause of perinatal morbidity and mortality in pregnant women. The predominant micro organisms isolated from vaginal swabs in pregnant women include both gram positive and gram negative micro organisms like Staph. aureus, beta-hemolytic Streptococci, E.coli and Klebsiella pneumonia [11].

Bacterial vaginosis (40 – 45 %), vulvovaginal candidiasis (20 – 25 %) and other infections account for about 15 – 20 % of vaginal infections. Most vaginal infections are caused by commensal bacteria of the female genital tract [12]. The vaginal ecosystem changes from time to time due to a variety of endo and exogenous factors [13].

Highest incidence of vaginal infections in our study was found in 26 – 30 years age group, followed by 31 – 35 years age group probably because this is the sexually most active age group. The higher incidence can also be due to estrogen effect which increases the susceptibility of vaginal epithelium to micro organisms [14].

Conclusion

Lactobacilli constitute the major vaginal flora in women during child bearing age and is helpful in maintaining the acidic pH of vagina so that organisms do not invade and cause disease. The natural history of abnormal vaginal flora in pregnancy is still poorly understood but that it can cause perinatal morbidity and mortality is a well known fact. So, for a healthy perinatal outcome of pregnancy, a vaginal swab culture is required in all pregnant women.

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Original Article

A Study of Uropathogens associated with Asymptomatic Urinary Tract Infection in Antenatal Patients

Siddhant H. Karnik*, Varsha A. Singh, Aditi S. Pandey*, Neetu K. Singh***

Author Affiliation
*Research Scholar,
** HOD, Department of Microbiology, MMIMSR, Mullana, Ambala.

Reprint Request
Siddhant Hemant Karnik, A/203, Chakreshwar Co-operative Housing Society, Nallasopara (w), Maharashtra 401203.
E-mail: sid061991@gmail.com

Abstract

Background: India is a developing country with large population of child bearing age group, majority from low socioeconomic status. Unaware of the possibilities of asymptomatic bacteriuria and its complications and often neglecting minor symptoms due to ignorance, lack of medical facilities; ultimately face antenatal, perinatal and postnatal complications. *Aim/Objectives:* This study aimed at uropathogens associated with asymptomatic urinary tract infection in antenatal patients. The objectives of the study were: To determine rate of Asymptomatic Bacteriuria (ASB) in antenatal patients. To identify the uropathogens causing Asymptomatic Bacteriuria and to find out their antimicrobial susceptibility pattern. *Methods:* Clean catch midstream urine specimens were collected from 300 women of any gestational age who attended the Department of Microbiology from April 2014- April 2015. Uncentrifuged urine samples were taken for wet mount preparation. Cultures were done using CLED agar at 37° C. Antimicrobial susceptibility test were assessed using CLSI guidelines on Mueller Hinton agar. *Results:* Out of 300 antenatal patients Asymptomatic bacteriuria was positive in 10% of Women. E. coli and Staphylococcus aureus were the most common pathogen isolated which were found to be sensitive to Ceftriaxone and Ciprofloxacin (100%, 100%) respectively. *Conclusion:* The major pathogen isolated among 30 positive cases of Asymptomatic Bacteriuria were E. coli and Coagulase negative staphylococcus. All isolates of E.coli and Coagulase negative staphylococcus were found to be highly sensitive with antimicrobial particularly Ceftriaxone and Ciprofloxacin. Hence, this study recommends for screening cases of Asymptomatic bacteriuria and timely management is necessary to prevent the complications associated with asymptomatic bacteriuria.

Keywords: Asymptomatic Bacteriuria; Pregnant.

Introduction

Urinary tract infection during pregnancy is the most common health problem caused mainly by the colonization of micro-organism involving lower urinary tract [1].

Asymptomatic bacteriuria (ASB) is defined as significant bacterial count(>10 micro- organisms or colony forming units present per millilitre) in the person without symptoms [2] and is present in approximately 5 – 10 % of the pregnant women [3]) and if untreated, it leads to the development of symptomatic cystitis and pyelonephritis in 50% of patient [4]. Infection may be complicated by low birth weight and prematurity, preeclampsia, maternal anaemia, amnionitis and intrauterine death [5]. Early treatment of bacteriuria not only could avert the occurrence of acute or chronic pyelonephritis, but it could also diminish the risk of prematurity

and perinatal mortality [6].

Thus it becomes mandatory to eradicate the root cause way before it leads to some inevitable complications. Since urinary tract infections are bacterial in origin, the role of microbiologist becomes even more important to isolate the respective causative organism so that appropriate antibiotics can be prescribed for the treatment. An important aspect attached to it is, despite of the importance of ASB in antenatal patient, not much studies have been carried out in the developing countries, which includes the ever progressing India as well. So, with respect to the very fact and keeping in mind the importance of the diagnosis of ASB the present study was aimed to evaluate the causative agents which are causing ASB in antenatal patient.

Material and Method

The study was conducted on 300 urine samples from antenatal patient without symptoms of UTI in Department of Microbiology from April 2014 to April 2015. The ethical clearance were taken from ethical committee. After the detailed clinical history, clean catch midstream urine samples were collected in sterile screw-capped, wide-mouth container. Uncentrifuged urine samples were subjected to wet mount for microscopic examination and cultured on CLED agar. The CLED agar was incubated at 37° C for 24 hours and the selected colonies were subjected to biochemical test and antibiotic susceptibility testing.

Result

Out of 300 urine sample processed, 30(10%) showed the growth on CLED agar. The macroscopic examination of culture positive urine specimen, 73.3% specimen were pale yellow in color, 16.6% were red and 10% were having red-brown color. Appearance include 60% cloudy specimen, 30% milky and clear 10 %. The microscopic examination of urine showed pus cells (80%) followed by red blood cells (16.6%) and calcium oxalate crystals (10%) in antenatal patients with ASB. Majority of bacterial isolate were Escherichia coli (52.5%) followed by Coagulase negative staphylococcus (22.5%), Staphylococcus aureus (12.5%), Klebsiella pneumonia (7.5%) and Enterobacter species (5%), in ASB antenatal case. Antibiotic susceptibility testing showed that Ceftriaxone and Ciprofloxacin were the most sensitive drugs against urinary tract infection followed by Gentamycin.

Table 1: Macroscopic and Microscopic Examination and Findings of Specimen in Antenatal Patients

Macroscopic examination of urine		Percentage
Color	Pale yellow	22(73.3%)
	Red	5(16.6%)
	Red-brown	3(10%)
Appearance	Cloudy	18(60%)
	Milky	9(30%)
	Clear	3(10%)
Microscopic examination	Pus cells	24(80%)
	Red blood cells	2(6.6%)
	Calcium oxalate crystals	3(10%)

Table 2: Distribution of Gram Positive and Gram Negative Bacterial Isolates

Microorganisms (N=40)	Total no. of microorganisms
Escherichia coli	21 (52.5)
Coagulase negative staphylococcus	09 (22.5)
Staphylococcus aureus	05 (12.5)
Klebsiella pneumonia	03 (7.5)
Enterobacter species	02 (5)

Table 3: Antimicrobial Susceptibility Patterns of Gram Negative and Gram Positive Bacteria of Antenatal Patients with ASB

Microorganisms	Gentamycin	Nitrofurantoin	Ceftriaxone	Tetracycline	Ciprofloxacin
Escherichia coli	19(90.47%)	7(33.3%)	21(100%)	15(71.42%)	21(100%)
Klebsiella pneumonia	2(66.66%)	0(0%)	3(100%)	0(0%)	3(100%)
Enterobacter species	2(100%)	0(0%)	2(100%)	0(0%)	2(100%)
Cons	8(88.88%)	2(22.22%)	9(100%)	1(11.11%)	2(22.22%)
Staphyloccusaureus	1(20%)	2(40%)	5(100%)	1(20%)	3(60%)

Discussion

The global prevalence of ASB in pregnancy is found to range from 2-11 %. In the present study 10% of the cases showed culture positivity which was in accordance with Agersew Alemu et al [7] from Northwest Ethiopia (10.4%), while higher positivity rate (16%), (13%), (11%) was shown by Humera Ansari et al [8] from Hyderabad, T. Jeyaseelan Senthinath et al [9] from Tiruchirappalli and S. Jain et al [10]) from Gujarat respectively in contrast to it S.V. Lavanya et al [11] from Visakhapatnam, J. Jayalakshmi et al [12] from Tamil Nadu and R Sujatha et al [13]) from Kanpur showed (8.4%), (7.4%) and (7.3%) respectively which was lower as epidemiological patterns vary from one country to the other and in different geographic areas in the same country and seasonal variation and climatic conditions also affect its prevalence.

ASB if ignored could lead to different risk factors like anaemia, preterm labour, low birth weight so, thorough screening of urine sample should be done which includes both macroscopic as well as microscopic examination.

In present study 73% antenatal patient were having pale yellow color of urine which indicates definite relation with asymptomatic bacteriuria followed by red color (17%) this is consistent with the previous study conducted by Alex et al [14] that demonstrated that pale yellow color of urine was the major macroscopic finding.

Appearance of urine sample is another parameter in gross examination. In present study 60% of antenatal patients were having cloudy appearance of urine sample which was in concurrence with the presence of leukocytes, bacteria and yeast. Urine sample showed 30% milky appearance in the current study which may be due to neutrophils which is in concurrence with the microscopic finding.

Microscopic examination of specimen in antenatal patients with ASB is associated with the color of urine sample which in order is associated with different complications. In present study 80% of patient were having pus cells this result was closely associated with the positive urine culture and development of ASB. This was in concordance with the Alex et al [14].

The presence of red blood cells in urine denotes hematuria. Ophori et al [15] in his study detected in 6% cases. In present study 6.6% of patients were having hematuria who showed positive results for ASB which may be due to patients of chronic renal disease.

For the accurate identification of causative organism culture is necessary. Causative organisms of asymptomatic bacteriuria in females are usually the commensal bacteria of the female genital tract and the bowel. Different determinants of virulence, such as presence of adhesins, stasis produced by gravid uterus, physiological and morphological changes that occur during pregnancy play a role in causation of UTI. [12,16,17,11]. The present study revealed Escherichia coli (52.5%) as the most isolated organism. This finding correlates with many studies in India and abroad. Jayalakshmi et al [12] (57.4%), Gayathree et al [17] (51.6%), had the similar findings. E. coli is the most common microorganism in the vaginal and rectal area. Because of anatomical and functional changes and difficulty of maintaining personal hygiene during pregnancy, may increase the risk of acquiring UTI from E. coli [18] Followed by Coagulase negative staphylococcus (22.5%) because Staphylococcus (Coagulase negative staphylococcus and Staphylococcus aureus) are common microorganism of skin in vaginal area, which is comparable to result by Agersew Alemu et al [7] (22.5%), Humera Ansari et al [8]) (19.05%) and Getachew Feredeet al [19]) (14.3%) followed by Staphylococcus aureus (12.5%) which is comparable to studies by Getachew Feredeet al [19] (28.6%), Agersew Alemu et al [7] (10%) and Tazebew D. et al [20] (10.7%) followed by Klebsiella pneumonia (7.5%) which is comparable to result by Agersew Alemu et al [7] (10%), Getachew Feredeet al [19] (14.3%) and Tazebew D. et al [20] (3.6%), and Enterobacter (5%) which is comparable to result by Agersew Alemu et al [7] (5%).

Antimicrobial Sensitivity Testing is necessary for appropriate treatment thereby potentiating the prognosis of the disease.

Antimicrobial Sensitivity Test of Escherichia coli, Coagulase negative staphylococcus, Staphylococcus aureus, Klebsiella pneumonia and Enterobacter species showed that Ceftriaxone, Ciprofloxacin and Gentamycin were the most sensitive drugs which is in concordance with Getachew Feredeet al [19] and Agersew Alemu et al [7]. The high sensitivity to these antibiotics may be due to their broad spectra on bacteria.

Conclusion

Study was conducted in the Department of Microbiology on 300 patients showing no symptoms of urinary tract infection, we excluded those patients showing symptoms of urinary tract infection, from

April 2014- April 2015. The rate of culture positivity was 10%. The Macroscopic and microscopic screening method of urine specimen showed that pale yellow color was most prevalent color of urine and pus cell were more predominant then other findings. *Escherichia coli* and Coagulase negative staphylococcus were the most isolated organism because *Escherichia coli* is the most common microorganism in the vaginal and rectal area and Staphylococcus (Coagulase negative staphylococcus and Staphylococcus aureus) is the common microorganism of the skin near vaginal area. Antibiotic sensitivity pattern showed that Ceftriaxone and Ciprofloxacin were the most sensitive drugs against the bacterial isolates. Henceforth concluded that microbiological diagnosis is very essential for better treatment so that appropriate antibacterial drug can be initiated on time in order to limit the worsening of condition and to prevent complications and to develop the anitibiotic policy so that the drug of choice can be used in case of emergency.

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Indian Journal of Anthropology	2	12000	1200
Indian Journal of Biology	2	4000	400
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Indian Journal of Forensic Medicine and Pathology	4	15500	1550
Indian Journal of Forensic Odontology	2	4500	450
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Indian Journal of Medical & Health Sciences	2	6500	650
Indian Journal of Obstetrics and Gynecology	2	7000	700
Indian Journal of Pathology: Research and Practice	2	11500	1150
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Original Article

In Vitro Activity of Tigecycline against Methicillin Resistant *Staphylococcus Aureus*

Mande T.H.*, Mangalkar S.M., Chincholkar V.V.**, Gohel T.D.***, Gaikwad V. V.***, Puri B.S.*****

Author Affiliation
*Resident,**Assoicate Professor,***Assistant Professor, Dept. of Microbiology, Government Medical College & Hospital, Latur, Maharashtra. Pin-431512.

Reprint Request
Trupti Mande, Resident, Dept. of Microbiology, Government Medical College & Hospital, Latur, Maharashtra. Pin-431512.
E-mail: mandeabhay673@gmail.com

Abstract

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) emerged with increasing resistance to most of the antimicrobial agents used for treatment of infections. The incidence of hospital acquired MRSA varies from 25% in western part of India, to 50% in South India. There is need of new antimicrobial agents, as increase in resistance and spread of multi drug resistance among many pathogenic species. Tigecycline is a newer glycylcycline antimicrobial agent very active against bacteria resistant to other classes of antibiotics, including the quinolones & betalactams. *Aims & Objective:* To evaluate in vitro activity of Tigecycline against Methicillin resistant *Staphylococcus aureus*. *Material and methods:* A total of 50 MRSA were isolated from various clinical samples of patients attending to tertiary care hospital. The organisms were identified by conventional method and antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as per CLSI guidelines. Tigecycline MIC was determined by using E strips according to manufacturer's instruction. *Result:* In our study all MRSA isolates were sensitive linezolid and teicoplanin. All MRSA isolates were inhibited by concentration of ≤0.5µg / ml of tigecycline. Most of strains in our study had MIC value less than 0.125µg / ml which was much below the US FDA cut offs for the susceptibility. *Conclusion:* Tigecycline is a potent anti microbial agent against MRSA. In the view of its excellent activity against MDR pathogens, it is prudent to reserve tigecycline for life threatening infections when other options fail.

Keywords: MRSA; Tigecycline; *Staphylococcus Aureus*; MDR Pathogens; E-Test.

Introduction

Significant changes in causative organism of nosocomial bacterial infections have been observed globally over past 100 yrs. In the first of half of 20th century, Gram positive organism particularly *Staphylococcus aureus & Streptococcus spp.* were of primary concern. By the end of 1970s, Methicillin resistant *Staphylococcus aureus* (MRSA) had also emerged with increasing resistance to most of the antimicrobial agent used for treatment of infections [1]. MRSA is now endemic in India. The incidence of hospital acquired MRSA varies from 25% in western part of India, to 50% in south India. Isolation of community acquired MRSA has also been identified & increasingly reported from India [2].

Resistance to currently available antibiotics is increasing at an alarming rate. At the same time, development of new antimicrobial agent to treat such serious bacterial infections is decreasing. As a result of the emergence and spread of multidrug resistance in many pathogenic species, the need for new antimicrobial agent is more urgent & greater than ever [3].

Tigecycline is a newer glycylcycline antimicrobial agent that induces its bacteriostatic effect by binding to high affinity intracellular site of the bacterial 30s

ribosome. Tigecycline is very active against bacteria resistant to other classes of antibiotics, including quinolones & betalactams. Tigecycline additionally, resist deactivation by most known tetracycline resistance mechanism [6]. Because of this promising profile against clinically important bacteria, as well as promising pharmacodynamic & pharmacokinetic data tigecycline is good alternative to treat MDR pathogens [3-5]. So considering multidrug resistance among MRSA, we tried to evaluate in vitro activity of tigecycline by E test.

Aims & Objectives

To evaluate in vitro activity of tigecycline against Methicillin resistant *Staphylococcus aureus* from various clinical samples by E test.

Material & Methods

A total of 50 MRSA were isolated from various clinical samples of patients attending to tertiary care hospital for period of three months. The strains were isolated from pus (n=36), blood (n=9) and urine (n=8). The organisms were identified by conventional method using Gram positive panel. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method using different antimicrobial agents; penicillin G [10 U], cefoxitin [30µg] gentamycin [10 µg], ciprofloxacin [5µg] erythromycin [15µg] clindamycin[2µg] Amoxycillin-clavulanic acid [20 / 10 µg] Levofloxacin [5 µg] linezolid [30µg] Teicoplanin [30µg] as per CLSI guidelines. Tigecycline [15µg] disc diffusion testing was done by using US FDA breakpoints, tigecycline MIC was determined by using E strips according to manufacturer's instruction.

Results and Observations

In our study, all MRSA isolates were sensitive to linezolid and teicoplanin. In the present study, 80 % of MRSA isolates were sensitive to levofloxacin while only 20 % were sensitive to Gentamycin and ciprofloxacin. (Table 1).

In our study, all MRSA isolates were inhibited by concentration of d"0.5µg / ml of tigecycline. Most of strains in our study had MIC value less than 0.125µg / ml which was much below the US FDA cut offs for the susceptibility (Table 2).

Table 1: Antimicrobial susceptibility pattern of MRSA isolates (n= 50)

Antimicrobial agent	S	I	R
Penicillin (10U)	0	0	100%
Cefoxitin (30µg)	0	0	100%
Gentamycin (10µg)	20%	0	80%
Ciprofloxacin (5µg)	20%	0	80%
Amoxycillin-clavulanic acid (20 / 10 µg)	0	0	100%
Erythromycin (15µg)	30%	0	70%
Clindamycin (2µg)	40 %	0	60 %
Levofloxacin (5 µg)	80%	0	20%
Lenozolid (30µg)	100%	0	0
Teicoplanin (30µg)	100 %	0	0
Tigecycline (15µg)	100%	0	0

S-sensitive, I-intermediate sensitive, R-resistant.

Table 2: Minimum inhibitory of concentration of MRSA strains for tigecycline obtained by E test

	MIC of tigecycline (ug/ml)		
	< 0.5 to 0.25	0.25-0.125	< 0.125
MRSA (n=50)	6	10	34

Discussion

Emergence of multidrug resistance among methicillin resistant *Staphylococcus aureus* strains have led to limited therapeutic options, resulting in increased morbidity and mortality. The development of new anti-microbial agents with novel modes of action is critically needed to keep in pace with development and spread of drug resistance mechanisms among bacteria [7].

Tigecycline is a glycylcycline compound with broad spectrum of bacteriostatic activity against Gram positive pathogens including MRSA [3]. It acts by inhibiting the 30s subunit of the ribosome.

Tigecycline does not exhibit co-resistance with known mechanisms of resistance. Its capacity to penetrate into various tissues makes it useful in the treatment of infections of skin and soft tissue as well intra abdominal infections [8-0].

In our study, 100 % strains were sensitive to linezolid and teicoplanin. Similar observations were shown by manisha mane et a [10].

In present study, 80 % strains were sensitive of levofloxacin similar result were obtained by Manoharan et al [7] while Shanthi M et al in his study reported only 25 % sensitivity to levofloxacin [11]. In comparison to our study Manisha mane et al reported somewhat higher sensitivity to ciprofloxacin and gentamycin [10].

In our study all strains were sensitive to tigecycline. Most of strains had MIC value less than 0.125µg / ml which was much below the US FDA cut offs for the susceptibility. Many other Indian and foreign studies reported MIC90 of tigecycline ranging from 0.125µg / ml to 0.5µg / ml against MRSA isolates[7, 12-14]. The activity of tigecycline against *Staphylococcus Aureus* is completely unaltered by the presence of methicillin or glycopeptides resistance genes. It is the most potent antimicrobial agent when tested against glycopeptides-intermediate resistant *Staphylococcus Aureus [15].*

To conclude, the present study shows tigecycline is a potent anti microbial agent against MRSA. In the view of its excellent activity against MDR pathogens, it is prudent to reserve tigecycline for life threatening infections when other option fails.

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Case Report

Isolation of Fluconazole Sensitive Stephanoascus Ciferrii in BAL Fluid from Renal Transplant Patient Presenting with Pneumonia

Sodani Sadhna*, Hawaldar Ranjana, Chowksey Anita**, Bhilware Hemlata****

Author Affiliation
*Assistant Professor, Microbiology, MGM Medical college, Indore.
**Sampurna Sodani Dagnostic Clinic, Indore.

Reprint Request
Ranjana Hawaldar,
Sampurna Sodani Diagnostic Clinic, LG-1, Morya Centre, Race Course Road, Indore, Madhya Pradesh.
E-mail: drranjana@sampurnadiagnostics.com

Abstract

Introduction: The introduction of newer immunosuppressive agents has led to a shift in the spectrum of infections occurring after kidney transplantation. This may be due to blunted inflammatory response in such patients and a timely diagnosis and institution of therapy is essential in such cases. Among fungal agents, Candida is the most commonly isolated species. A new teleomorphic species of Candida, Stephanoascus ciferrii has been associated with systemic mycosis in immunocompromised hosts. This species is particulary resistant to fluconazole. Here we report a case of fluconazole sensitive Stephanoascus ciferrii isolated from BAL Fluid in a kidney transplant patient. *Materials and Methods:* BAL Fluid was inoculated on Blood and MacConkey agar plates and for fungus isolation on Saboraud's Agar. After 24 hrs of incubation at 37^0C, Blood and MacConkey agar plates showed growth. Growth was also observed on Saboraud's Agar. Gram's staining of growth from MacConkey Agar showed growth of gram negative bacilli and from Saboraud's Agar showed growth of budding yeast forms suggestive of Candida species . For identification and susceptibility of these organisms Gram negative panel and YST panel was selected and performed on Vitek II (Biomerieux). *Results:* The bacteria were identified as Klebsiella Pneumoniae and yeast was identified as Stephanoascus Ciferrii. *Conclusion:* Candida ciferrii or Stephanoascus ciferrii as it is known is a new strain of Candida, which has rarely been associated with human infection. However it can cause opportunistic infection in immuno compromised patients and a high index of suspicion is required for a correct diagnosis to be made.

Keywords: CKD; Stephanoascus Ciferrii; Renal Transplant; Fluconazole.

Introduction

The introduction of newer immunosuppressive agents has lead to a shift in the spectrum of infections occurring after kidney transplantation. This may be due to blunted inflammatory response in such patients and a timely diagnosis and institution of therapy is essential in such cases. Infections are a major cause of morbidity and mortality in patients of kidney transplant. The major infections in kidney transplant patients range from bacterial, viral, tuberculosis and fungal infections. Among fungal agents, Candida is the most commonly isolated species. A new teleomorphic species of Candida, Stephanoascus ciferrii has been associated with systemic mycosis in immuno compromised hosts [1]. This species is particulary resistant to fluconazole.

Here we report a case of fluconazole sensitive

Stephanoascus ciferrii isolated from BAL Fluid in a kidney transplant patient.

Case Report

A 45 year of old female, diabetic patient with history of Koch's renal transplant (spousal) presented with progressive increase in dyspnoea and cough with copious mucopurulent expectoration for 2 days . On general examination there was tachypnea (respiratory rate 35/min) with cyanosis.

Respiratory system examination showed decreased breath sounds and rhonchi all over the chest. She had a case of new onset diabetes after transplantation secondary to Tacrolimus (NODAT) and had CKD Grade 5 since last 7 years. So she had received a kidney transplant and was on triple maintenance a Immunosuppressive therapy.

She was admitted to ICU and a battery of blood tests including blood culture and fungal culture were sent to our diagnostic Centre for evaluation. Tests for CMV and Pneumocystis carinii infection werenegative. Meanwhile she was put on immunosuppressive drugs and Inj.Meropenem and Levofloxacin was started empirically..

Chest X-ray showed mild accentuation of marking. Her blood parameters are shown in Table 1.

Bronchial alveolar lavage was performed and BAL Fluid was sent for culture and routine examination. Gram's Stain showed budding yeast cells and Gram negative bacilli. Z.N Stain was negative for Acid fast bacilli.

BAL Fluid was inoculated on Blood and MacConkey agar plates and for fungus isolation on Saboraud's Agar. After 24 hrs of incubation at 37^{0}C, Blood and MacConkey agar plates showed growth. Growth was also observed on Saboraud's Agar. Gram's staining of growth from MacConkey Agar showed growth of gram negative bacilli and from Saboraud's Agar showed growth of budding yeast forms suggestive of Candida species (figure1). For identification and susceptibility of these organisms Gram negative panel and YST panel was selected and performed on Vitek II (Biomerieux). The bacteria was identified as Klebsiella Pneumoniae and yeast was identified as Stephanoascus Ciferrii. Susceptibility pattern of both organisms is shown in Table 2 and 3.

Microscopic examination of fungal colonies showed extensive branches and blastoconidia ,oval chains of different sizes, arranged along pseudohyphae and true hyphae. The confirmation of S. Ciferrii was done through automated Vitek II system (Biomerieux).

Based on the culture reports, Fluconazole was started for fungal infection and the antibiotics were continued for Klebsiella pneumoniae. She responded well to the treatment and was discharged from the hospital at a creatinine of 1.8 mg%.

Table 1: Investigative findings

Investigations CBC	1st day	3rd day	5th day
Hb gm%	10.9	12.6	12.6
RBC$10^{6/}$uL	3.31	3.78	3.73
PCV%	32.8	38.3	38.5
Total WBC count	2.2	2.4	4.7
10^3/uL	82	46	82
Neutrophils %	14	45	14
Lymphocytes %	02	03	02
Monocytes %	02	06	02
Eosinophils %	241	433	163
Platelets 10^3/uL	1.84	1.80	1.80
Creatinine mg%			

Table 2: Drug sensitivity report of Bronchial Lavage

Organism : Klebsiella Pneumoniae

Drugs	MIC	Interpretation
Amikacin	<=2	S
Amoxicillin		R
Ampicillin	>=32	R

Amox/K Clav	16	I
Cefepime	>=64	R
Cefoperazone/Sulbactum	16	S
Cefotaxime		S
Ceftriaxone	>=64	R
Cefuroxime	>=64	R
Cefuroxime Axetil	>=64	R
Ciprofloxacin	>=4	R
Colistin	< 0.5	S
Ertapenem	<=0.5	S
Gentamicin	<=1	S
Imipenem	<=0.25	S
Meropenem	<=0.25	S
Pip/Tazo	64	I
Tigecycline	<=0.5	S
Trimethoprim/Sulfamethoxazole	320	R

Table 3: Fungal Culture in Bronchial Lavage

Organism- Stephanoascus ciferrii		
Drugs	**MIC**	**Interpretation**
Fluconazole	<=8	S
Voriconazole		
Caspofungin	>=4	
Micafungin		
Amphotericin B	8	R
Flucytosine	<=1	S

Fig. 1: Colonies of Stephanoascus ciferrii in Saboraud's agar

Discussion

S.ciferrii was first identified by Smith et al in 1976 [1]. In humans, infection by S.ceferrii is on the rise and is associated with ear infections, non-insulin dependent diabetes mellitus, vascular disorders, val vular heart disease and mostly with cases of onychomycosis [2,3,4]. There are reported cases of infection with S.ciferrii in immune compromised patients[5,6,7].

The prognosis of infections caused by S.ciferrii is good especially in otitis patients. However in immuno compromised patients, it is an opportunistic pathogen as is the case with our patient. However most of the cases show resistance to fluconazole and miconazole [8]. There are few reported cases of S.ceferrii infection resistant to fluconazole. Kaushik Shah et.al in 2013 reported a case of fluconazole sensitive Candida ciferrii infection in a diabetic COPD patient presenting with pneumonia [9].

Candida is a part of normal flora of the oropharynx and GIT, so growth of Candida from upper respiratory samples is frequently considered to be a contamination. Although Candida species can be

isolated from Bronchial washings, tracheal aspirates and the BAL samples of patients but accompanying lung parenchymal invasion is rarely found. Isolation of Candida species with lung parenchymal involvement proves the pathogenecity of the organisms.

Conclusion

Candida ciferrii or Stephanoascus ciferrii as it is known, is a new strain of Candida, which has rarely been associated with human infection. However it can cause opportunistic infection in immuno compromised patients and a high index of suspicion is required for a correct diagnosis to be made.

Conflict of Interest

None

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Case Report

A Case Report of Meningococcal Septicaemia in a 2 Year Old Child

Panicker S.S.*, Mangalkar S.M., Gohel T.D.***, Gaikwad V.V.***, Puri B.S.***, Chincholkar V.V.****

Author Affiliation
*Resident,**Associate Professor,***Assistant Professor, Dept. of Microbiology, Government Medical College & Hospital, Latur, Maharashtra. Pin-431512.

Reprint Request
Panicker Sreejith Sreedharan, Resident, Dept. of Microbiology, Government Medical College & Hospital, Latur, Maharashtra. Pin-431512.
E-mail: shreeaks@gmail.com

Abstract

Acase of meningococcal septicemia in a male child was reported in Nov. 2014 at Government Medical College, Latur. The main features were fever with rash, respiratory distress since 4 days and altered sensorium since 2 days. The rash was purpuric and present all over the body including palms and soles. Gram stain from purpuric sites showed Gram negative diplococci suggestive of *Neisseria meningitidis.* In case of meningococcal disease, some patients develop acute meningococcemia whilst others develop meningitis. The mechanism is unknown, but the case fatality of acute meningococcemia is tenfold than that of meningococcal meningitis. It is possible that the delay in diagnosis of meningococcal disease with a maculo-papular rash alone might contribute to mortality as these children are thought to have viral illnesses and are not started on antibiotic treatment. Thus it is important to recognize this life-threatening condition in the initial stable phase to provide the emergency management so as to reduce mortality.

Keywords: Maculo-Papularrash; Meningococcemia; Neisseria meningitidis.

Case Report

A 2 years old male child was brought by parents with chief complaints of altered sensorium since 2 days and respiratory distress since 4 days. Child was well about 7 days back to start with fever without any association of chills and rigors, 2 days after fever child developed rash that appeared first on abdomen and gradually spread to hand, face, palm and soles. He was put on some medicines and then he developed respiratory distress and was referred to physician who felt need of admission. After admission he developed tonic-clonic seizures that lasted for 15min followed by unconsciousness for half an hour, the sensorium was deteriorating and the child remained unconscious for 24 hrs with increase in respiratory rate and was referred to our institute.

Child had past history of pneumonia 5 months back and was admitted in private hospital for 5 days. No significant birth history and immunization followed as national immunization schedule.

Physical examination showed that there was generalized maculopapular rash on the abdomen, palm, soles, face and limbs (Fig.1). Sub occipital lymph node was negative. There was no neck rigidity. Chest, cardiac and abdominal examination did not reveal any abnormality.

Laboratory Diagnosis

After initial investigations, multiple slit skin smears were taken from the sites of purpura and were stained with Gram's stain. They showed plenty of epithelial cells and neutrophils with gram negative diplococci. CSF microscopy showed 10cell/cumm

with predominant lymphocytes. CSF sugar and proteins were normal.Further investigations showed white cell count of 10500 with neutrophilia; platelet count were reduced to 54,000/cu.mm; D-Dimer was significantly raised (10,400).

5ml of blood was taken and inoculated into the Brain Heart Infusion broth. Also, fluid from the slit skin smear of the purpuric sites wasdirectly inoculated onto Blood agar and Chocolate agar. (All microbiological investigations including collection of sample for culture and inoculation onto appropriate media were done by the bedside under aseptic conditions) Both blood culture and culture from purpuric lesion grew organism which was catalase and oxidase positive, fermented glucose and maltose and Gram stain showed Gram negative diplococci suggestive of *Neisseria meningitidis* (fig. 2-3), which was sensitive to cefotaxime, ceftriaxone, ciprofloxacin. He was subsequently transferred to ICU for treatment. Intravenous antibiotic including ciprofloxacin and cefotaxime were administered.The diagnosis was established as Purpura Fulminans following meningococcal-septicaemia. The patient expired within 48 hours of admission to our institute. Post mortem was not done as the diagnosis was well established.

Fig. 1: 2year old boy with extensive purpuric rashes.

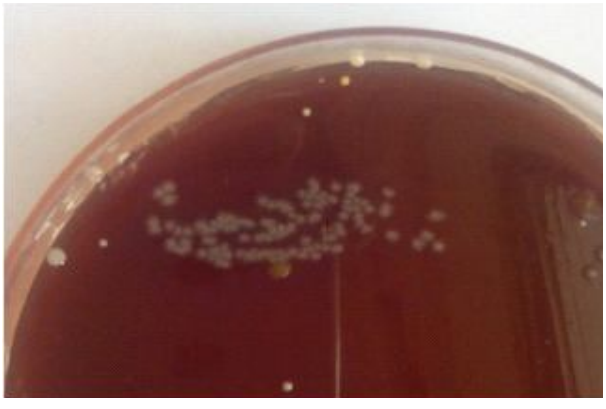

Fig. 2: Colonies of *N.meningitidis* grown from blood culture.

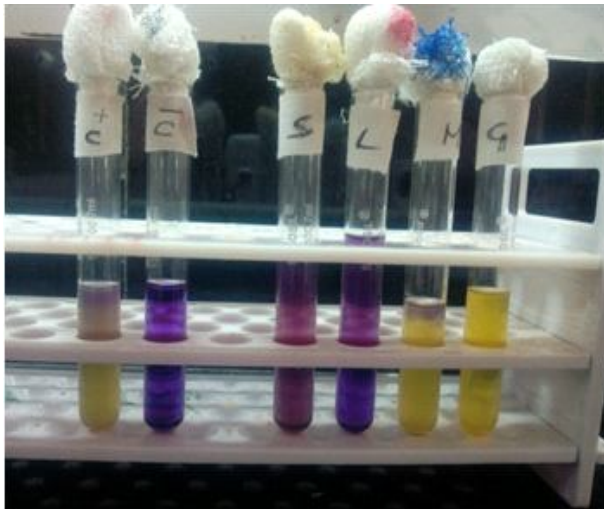

Fig. 3: Sugar Fermentation test.
C+ = Positive control: C- = Negative control
G= Glucose: M = Maltose: L = Lactose: S= Sucrose
Figure showing fermentation of glucose and maltose but not of Lactose and Sucrose as compared to positive and negative control.

Discussion

In meningococcal infections, some patients develop acute meningococcemia whilst others develop meningitis. The mechanism is unknown, but the case fatality of acute meningococcemia is tenfold than that of meningococcal meningitis, which is generally less than 5%. The initial treatment for both conditions is the same [1].

Maculopapular rash alone in meningococcal infection has also been reported in fatal cases and it is possible that the delay in diagnosis of meningococcal disease with a maculopapular rash alone might contribute to mortality as these children are thought to have viral illnesses and are not started on antibiotic treatment [2,3]. Although the maculopapular rash is the distinctive sign of menin-gococcal infection, it is seen in only 7% of cases.The rash may rapidly evolve into prominent petechiae and purpura and may progress to purpura fulminans, a necrosis of the skin and under-lying tissues due to thrombosis [4].

Purpura Fulminans (PF) is a hematological emergency in which there is skin necrosis and disseminated intravascular coagulation. This may progress rapidly to multi-organ failure caused by thrombotic occlusion of small and medium-sized blood vessels. PF may complicate severe sepsis or may occur as an autoimmune response to otherwise benign childhood infections [4]. PF may also be the presenting symptom of severe heritable deficiency of the natural anticoagulants protein C or protein S

[5,6]. Early recognition and treatment of PF is essential to reduce mortality and to prevent major long-term health sequelae. However, management strategies require accurate identification of the underlying cause [5].

Conclusion

The aim of this case report is to emphasize the importance of bed side diagnostic microbiological procedures like slit skin smear and Gram stain which helped us in the prompt diagnosis and treatment of the underlying cause (meningococcal septicaemia).

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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.

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Article in supplement or special issue

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Corporate (collective) author

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Unpublished article

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Personal author(s)

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Chapter in book

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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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