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Original Article

Prevalence and Identification of Streptococci and Enterococci from Various Clinical Specimens in a Tertiary Care Centre

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Abstract

Aim: *To study the distribution of Streptococci and Enterococci in various clinical specimens.*

Materials and Methods: *Streptococci and Enterococci isolated from various clinical specimens in Central Microbiology Laboratory, Government Medical College, Thiruvananthapuram over a period of one year from January 2014 to December 2014 were included in the study. The specimens were cultured using aerobic and anaerobic microbiological techniques. Streptococci and Enterococci were identified by standard laboratory procedures as per CLSI guidelines. Antimicrobial susceptibility testing was carried out using disc diffusion method. Grouping was done by latex agglutination.*

Results: *Majority of group A Streptococci (GAS-82.95%), group B Streptococci(GBS-72.60%),group F Streptococci (GFS-63.63%) and group G Streptococci (GGS-50%) were obtained from pus .Group C Streptococci(GCS-58.33%) was obtained from sputum. Maximum number of Enterococci was obtained from urine(45.37%).Majority of the isolates of GAS(52.27%),GBS(49.31%),GCS(41.66%)and GGS(50%) were from skin and soft tissue infections while GFS(36.36%) was from dentoalveolar abscess. Theenterococcal isolates were mainly from urinary tract infections(44.44%).All the Streptococcal isolates were sensitive to Penicillin. Only 96.29% of Enterococci were sensitive to Vancomycin.*

Conclusion: *Most of the Streptocccal infections are life threatening. Hence it is necessary to develop preventive, diagnostic and therapeutic strategies against them.Due to the emergence of Vancomycin resistant Enterococci, it is recommended to perform screening test and MIC determination to confirm resistance of local strains to this antibiotic.*

Keywords: *Streptococci, Enterococci, Vancomycin resistant Enterococci.*

Introduction

Streptococci is the general term for a diverse collection of gram positive cocci that are typically seen as chains or pairs. [1] They are known to cause a diverse array of human infections. Group A Streptococci (GAS) constitute the most important and prevalent members of this group, causing clinical syndromes ranging from self-limited pharyngitis and impetigo to potentially life-threatening toxic shock syndrome.[2]Group B streptococci (GBS) are a leading cause of infections in neonates and pregnant women and also cause invasive diseases in children and non-pregnant adults. Group C (GCS) and group G (GGS) streptococci are usually commensals of the pharynx, skin, gastrointestinal tract, and female genital tract. Penicillin is the drug of choice for the treatment of streptococcal infections. Erythromycin can be used in patients allergic to Penicillin. Aminoglycosides are often used in combination with Penicillin for the treatment of bacterial endocarditis caused by Streptococci.

Over the past few years the occurrence of antimicrobial resistance among Enterococci especially high level resistance to aminoglycosides and resistance to Vancomycin has been increasingly reported. Since VRE have an intrinsic resistance to most of the commonly used antibiotics and the ability to acquire resistance to most of the currently available antibiotics,they pose a major therapeutic challenge.[3,4]

Materials and Methods

A descriptive study was conducted in Government Medical College, Thiruvananthapuram for a period of six months to know the prevalence of Streptococci and Enterococci in clinical samples. All the samples that came to the Central Microbiology lab during this period namely urine, pus, blood, sputum and body fluids were included in the study. These samples were obtained from conditions like bone and joint infections, tonsillitis, dentoalveolar abscesses, pneumonia, urinary tract infections, septicaemia, skin and soft tissue infections and peritonitis.

All the specimens except urine and blood were inoculated on blood agar,chocolate agar and Mac Conkeysagar. Urine was inoculated on blood agar and Mac Conkeys agar only. After inoculation the media were incubated aerobically and anaerobically for 24-48 hours. Brain heart infusion broth was used as primary medium for all blood samples. After 6 hours of incubation subcultures were done on blood agar and Mac Conkeyagar. Growth was recorded on the next day.

Presumptive identification of Streptococci was done based on the colony characteristics, type of haemolysis, gram staining, catalase negativity and Bacitracin sensitivity. Identification of Enterococci included gram staining, catalasenegativity, growth on 6.5% sodium chloride, aesculin hydrolysis and heat resistance.

Antimicrobial susceptibility test was done on blood agar using Kirby Bauer disc diffusion method as per the CLSI guidelines. Penicillin (10 IU), Erythromycin (15µg) and 1st generation Cephalosporins (30µg) were tested for all groups of Streptococci. For Enterococci Ampicillin (10µg), Erythromycin (15µg), Gentamicin (120µg) and Vancomycin (30µg) were tested. Enterococcus strains that were resistant to Vancomycin by disc diffusion method were tested for MIC using Vancomycin E strip.MIC for Vancomycin greater than 32µg/ml was considered as resistant.

Latex agglutination test (Himedia Labs, India) was used for the identification of Streptococcal groups A,B,C,F, G and also Enterococci. These antigens were extracted from the cell wall in liquid form and reacted with group specific antibodies.

Results

Of the 317 isolates 209 (65.93%) were Streptococci and 108 (34.06%) were Enterococci (Figure 1). Most of the streptococcal isolates belonged to group A (42.10%) (Table 1).

Maximum number of group A (82.95%), B (72.60%), F(63.63%),G (50%) and ungroupable Streptococci (57.14%) were obtained from pus. Most of the GCS was obtained from sputum (58.33%)(Table 2). Enterococci was obtained mainly from urine(44.44%)(Table 3).

Table 4 shows the isolation of Streptococcifrom various clinical conditions. Most of the Enterococci (45.37%) were from urinary tract infection (Table 5)

All the streptococcal isolates were sensitive to Penicillin and cephalosporin.75% of GAS, 54.79% of GBS, 83.33% of GCS and 76.19% of ungroupable Streptococci were sensitive to Erythromycin while all GFS and GGS were sensitive to the same.All GAS,83.33% of GCS and 25% of GGS were sensitive to Bacitracin. (Table 6).75.92% of Enterococci were sensitive to Ampicillin and Erythromycin,86.11% to Gentamicin and 96.29% to Vancomycin.(Table 7).

4.3% of cases from whom streptococcal isolates were obtained and 1.85% of cases from whom enterococcal isolates were obtained had a fatal outcome.

Figure 1: Distribution of isolates.

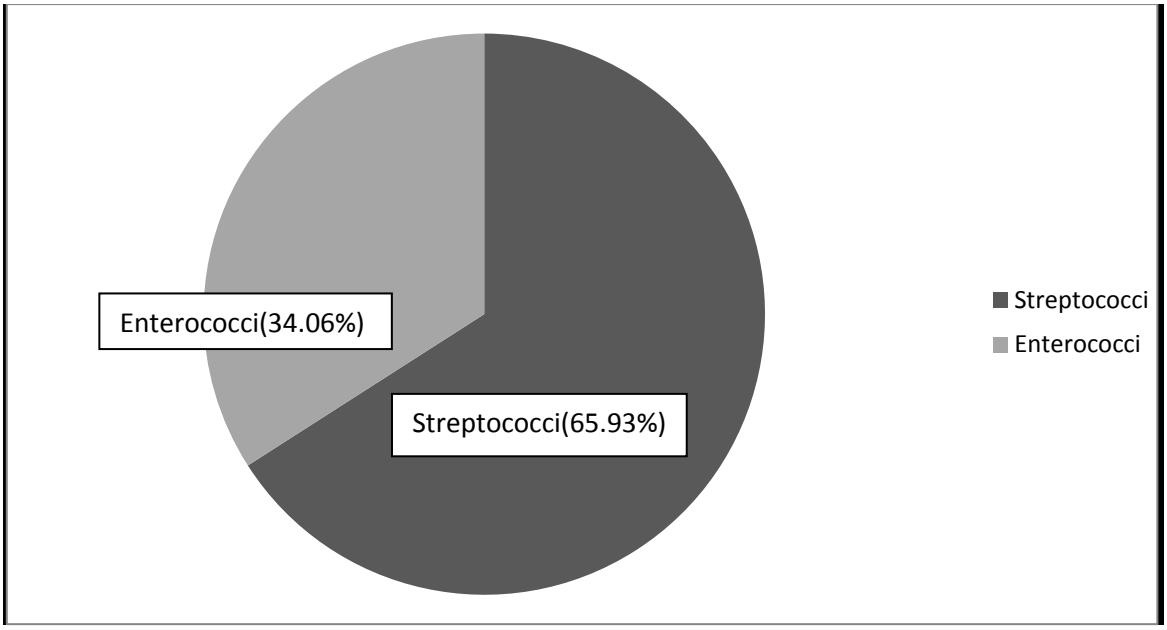

Table 1:Distribution of Streptococci

Group	Number	Percentage
A	88	42.10
B	73	34.92
C	12	5.74
F	11	5.26
G	4	1.91
Ungroupable	21	10.04

Table 2: Distribution of Streptococci from clinical samples.

	Pus		Urine		Blood		Sputum		Body fluids		Total
Isolates	No:	(%)	No:	(%)	No:	(%)	No:	(%)	No:	(%)	
A	73	82.95	7	7.95	0	-	5	5.68	3	3.40	88
B	53	72.60	11	15.06	1	1.36	4	5.47	4	5.47	73
C	5	41.66	0	-	0	-	7	58.33	0	-	12
F	7	63.63	3	27.27	0	-	1	9.09	0	-	11
G	250		0	-	0	-	1	25	1	25	4
Ungroupable	12	57.14	6	28.57	2	9.52	0	-	1	4.76	21
Total	152		27		3		18		9		209

Table 3: Distribution of Enterococci from clinical samples

Clinical sample	Enterococci	
	Number	Percentage
Pus	44	40.74
Urine	48	44.44
Blood	8	7.40
Sputum	1	0.92
Body fluids	7	6.48

Table 4: Distribution of Streptococciobtainedfrom various clinical conditions

Infections		A	B	C	F	G	Ungroupable	Total
Bone and joint infection	No:	7	3	0	1	0	0	11
	%	7.95	4.10	0	9.09	0	0	
Tonsillitis	No::	9	8	3	1	0	4	25
	%	10.22	10.95	25	9.09	0	19.04	
Dentoalveolar abscess	No:	13	7	0	4	0	0	24
	%	14.77	9.58	0	36.36	0	0	
Pneumonia	No:	6	4	4	0	0	0	14
	%	6.81	5.47	33.33	0	0	0	
UTI	No:	7	12	0	2	1	5	27
	%	7.95	16.43	0	18.18	25	23.80	
Septicaemia	No:	0	1	0	0	0	1	2
	%	0	1.36	0	0	0	4.76	
Skin and soft tissue infection	No:	45	36	5	3	2	10	101
	%	52.27	49.31	41.66	27.27	50	47.61	
Peritonitis	No:	1	2	0	0	1	1	5
	%	1.13	2.73	0	0	25	4.76	
Total		88	73	12	11	4	21	209

Table 5: Distribution of Enterococci obtained from various clinical conditions

Clinical condition	Enterococci	
	Number	Percentage
Bone and joint infection	1	0.92
Tonsillitis	3	2.77
Dentoalveolar abscess	0	0
Pneumonia	3	2.77
Urinary tract infection	49	45.37
Septicaemia	7	6.48
Skin and soft tissue infections	42	38.88
Peritonitis	3	2.77

Table 6: Antibiotic sensitivity pattern of Streptococci (percentage sensitivity)

Groups	No:	Penicillin (10 IU)	1st gen Cephalosporins (30µg)	Erythromycin (15µg)	Bacitracin (0.04units)
A	88	100%	100%	75%	100%
B	73	100%	100%	54.79%	0%
C	12	100%	100%	83.33%	83.33%
F	11	100%	100%	100%	0%
G	4	100%	100%	100%	25%
Ungroupable	21	100%	100%	76.19%	0%

Table 7: Antibiotic sensitivity pattern of Enterococci(Percentage sensitivity)

Antibiotic	Enterococci (108)	
	Number	Percentage
Ampicillin(10µg)	82	75.92
Erythromycin(15µg)	82	75.92
Gentamicin(120µg)	93	86.11
Vancomycin(30µg)	104	96.29

Discussion

Streptococci are significant human pathogens causing a wide spectrum of diseases ranging from uncomplicated infections to serious life threatening diseases. In the present study, out of 209 Streptococci isolated 42.1% were group A Streptococci,34.92% group B Streptococci,5.74% group C Streptococci,5.26% group F Streptococci, 1.91% group G Streptococci and 10.04% were ungroupable. Enterococciformed 34.06% of the isolates. Studies done by Rantala S et al and Schattner A et al showed that GAS formed 29.9% and 33%,GBS formed 24.4% and 49%,GCS formed 5.73% and 6%,GGS formed 40.76% and 10% of their studies respectively showing higher rates of isolation than the present study.[5,6]

In this study out of 88 GAS,52.27% were from skin and soft tissue infections,7.95% were from bone and joint infections. Lamagni TL et al and Davies HD et al also claim skin and soft tissue infections as the most common manifestation of GAS infection-42%and 48% respectively.[7,8] Study done by Le Hello et al at new Caledonia in 2006 showed the percentage of isolation of GAS from skin and soft tissue as 71.1% and from bone and joint infection as 9.97% which is higher than that obtained in the present study.[9]

In the present study out of 73 GBS,49.31%were from skin and soft tissue infections,16.43%from urinary tract infections and10.95% from tonsillitis. Studies conducted by Lee et al and Farley et al also showed skin and soft tissue infections as the most common presentation of invasive GBS infection.[10,11]

Out of the 12 isolates of GCS, 41.66% were from skin and soft tissue infections. Mohanty et al, AIIMS 2004 reports a case of Streptococcal bacteremia caused by GCS from a 10 year old male patient with a known history of aplastic anaemia.[12]GFS was isolated from 11 cases in the present study,of which four(36.36%) were from dentoalveolar abscess. A study by Dr.Sarada Devi, Government Medical College Thiruvananth-apuram in 1994 reported isolation of GFS from 2 cases of liver abscesses and 2 cases of brain abscesses.[13] The number of GGS isolated in the present study was 4 of which two (50%) were from cases of skin and soft tissue infections. In the study conducted by Dr Sarada Devi GGS was isolated from cases of cellulitis, wound infection, abscess and trophic ulcer.[13]

In the present study ,out of 108 isolates of Enterococci 44.44% were from urine and 40.74% were frompus. Studies done by Jadan et al, Desai PJ et al showed that maximum Enterococcal isolates were from urine (40.30%,48.12%) followed by pus (31.90%,29.15%).[14,1]

Bacitracin susceptibility is a commonly used test for presumptive identification of *Streptococcus pyogenes*. In the present study all GAS were sensitive to Bacitracin.83.33% of GCS and 25% of GGS were also susceptible to Bacitracin. A study conducted by Menon T reported that 9.8% of GAS strains were resistant to Bacitracin, whereas 85.7% of GGS and 90% strains of GCS were sensitive to Bacitracin.[6]

In the present study all GAS were sensitive to Penicillin and 75% were sensitive to Erythromycin. This almost correlates with the studies done by Jain A et al and Lloyd C A et al in which all the GAS isolates were susceptible to Penicillin and 10.2% and 16.2% showed Erythromycin resistance.[17,18]

All the GBS were sensitive to Penicillin in the present study.54.79% of them were sensitive to Erythromycin. A study conducted by Hsueh P R et al stated that 6% of GBS were resistant to Penicillin and 46% were resistant to Erythromycin.[19]

In the present study all GCS were sensitive to Penicillin but only 83.33% were sensitive to Erythromycin .All GFS and GGS were sensitive to Penicillin as well as Erythromycin. In a study done by Broyles L N et al on antibiotic susceptibility testing of GCS and GGS there was no resistance to Penicillin but Erythromycin resistance was noted in 29% cases.[20]

Among the isolates of Enterococci75.92% were sensitive to Ampicillin and Erythromycin,86.11% were sensitive to Gentamicin and 96.29%were

sensitive to Vancomycin in the present study. Thus the prevalence of high level Gentamicin resistance (HLGR) was 13.88% and Vancomycin resistance was 3.71%.This is different from a study conducted by Mendiratta et al in a rural Hospital in Central India where HLGR was 46%.[21] Studies from Lady Hardinge Medical College, New Delhi, Chandigarh and Mumbai reported the percentage of Vancomycin resistant Enterococci as 8%,5.5% and 23% respectively.[22]

Conclusion

Streptococci contribute to a wide spectrum of infections in our hospital. Since most of these infections are life threatening, there is a need to develop preventive, diagnostic and therapeutic strategies against them.In the context of rising concern about the emergence of Vancomycin resistant Enterococci, it is recomended to perform screening test and MIC determination to confirm resistance of local strains to this antibiotic.

References

1. M.Kilian.*Streptococcus* and *Enterococcus.* Greenwood, Slack, Peutherer, Barer Medical Microbiology. 7th edition.Chapter 16. Philadelphia: Churchill Livingstone Elsevier; 2007.Page 178.
2. Musser JM, Shelburne SA (2009) A decade of molecular pathogenomic analysis of group A *Streptococcus*. J Clin Invest 119: 2455-2463.
3. Mandell GL,Bennet JE,Dolin R.Mandell, Douglas and Bennet's Priciples and practice of infectious diseases.2010; 7th edition, vol 2:2591-2686.
4. Borriello SP, Murray PR, Funke G. Topley and Wilson's Microbiology and microbial infections.10th edition, Baltimore: Hodder Arnold;2007 vol2:771-881.
5. RantalaS,Varikila V J,Vuento R et el. Clinical presentations of beta-haemolytic Streptococcal bacteremia:A population based study. Clin Microbiol Infect.2009 Mar;15(3):286-8.
6. Schattner A,Vosti K L.Bacterial arthritis due to beta-haemolytic *Streptococci* of serogroups A,B,C,F and G.Analysis of 23 cases and review of the literature. Medicine (Baltimore).1998 Mar;77 (2):122-39.
7. LamagniTL,DarenbergJ,HarariBL.Epidem iology of severe Streptococcal pygenes disease in Europe. Clin Microbiol.2008 July;46(7):2359-2367.
8. DaviesHD,Mc Geer A,Schwartz B et al.Invasive group A streptococcal infectins in Ontario, Canada. Ontario Group A Streptococcal study group. N Engl J Med.1996;335(8):547-54.
9. LeHelloS, Doloy A,Baumann F.Clinical and Micrbial characteristics of invasive *Streptococcal pyogenes* disease in new Caledonia,a region in Oceania with high incidence of acute rheumatic fever.JClin Microbiol.2010 May;48(5):1993.
10. Lee NY,YanJJ,Wu JJ et al.Group B streptococcal soft tissue infections in non-pregnant adults. Clin Microbiol Infect.2005;11:577-579.
11. FarleyMM.Group B Streptococcal disease in non pregnantadults.Atlanta Clinical Infectious Diseases.2001;33:556-61.
12. MohantyS, KapilA, Mohapatra M et al.Group C streptococcal bacteremia:Acase report from India.South east Asian J Trop Med public health.2005;35(4):877-8.
13. Dr Sarada Devi.A bacteriological study of Streptococcal infections. A study conducted in the Department of Microbiology, Government Medical College, Thiruvananthapuram;1994.
14. Jadan S and JayakumarK.Prevalence of *Enterococcus* species from various clinical specimens in ShriSathyaSai Medical College and Research Institute with special reference to speciation and their resistance to Vancomycin. International Journal of Medical and Clinical Research. 2012;3(4):154-160.

15. Desai PJ, Pandit D, Gogate MA. Prevalence, identification and distribution of varius species of *Enterococci* isolated from clinical specimens with special reference to urinary tract infection in catheterisedpetients. Indian Journal of Medical Microbiology(2001);19((3):132-137.
16. MenonT,LloydC,JacobS.ShouldBacitracin sensitivity be used in the presumptive identification of group A *Streptococci*? Indian Journal of Medical Microbiology. 2005;23(4):275.
17. Jain A,ShuklaVK,Tiwari V et al.Antibiotic resistance pattern of group A beta haemolytic*Streptococci* isolated from nrth Indian children. Indian J Med Sci.2008 Oct;62(10):392-6.
18. Lloyd CA,JacobSE,MenonT.Antibiotic resistant beta haemolytic *Streptococci*. Indian J Paediatr.2007 Dec;74(12):1077-80.
19. HsuehPR,TengLJ,Luh KT etal.High incidence of Erythromycin resistance among clinical isolates of *Streptococcus agalactiae* in Taiwan.Antimicrob Agents Chemther.2001;45(11):3205.
20. Broyles LN, Beneden CV,Beall B et al.poulation based study of invasive disease due to beta haemolytic*Streptococci* of groups other than A and B.Clinical infectious diseases.2009;48:706-12.
21. Mendiratta DK, Kaur H, Deotale V, Thamke DC, Narang R, Narang P. Status of high level aminoglycoside resistant Enterococcus faecium and Enterococcus faecalis in a rural hospital of central India. Indian J Med Microbiol. 2008 Dec;26 (4):369– 71. 11. Srivastava P, Mehta R, Nirwan PS, Sharma M, Dahiya
22. Sood S,MalhotraM,Das BK et al. Enterococcal infections and antimicrobial resistance.Indian J Med Res128.August 2008:111-121.