DOI: NJLM/2015/11684:2031

Original Article

Microbiology Section

Isolation, Identification and Speciation of *Enterococci* by Conventional Method and their Antibiogram

SHANMUKHAPPA, D VENKATESHA, AJANTHA G S, AMRUTHA KUMARI B, MALLIKARJUN KOPPAD

ABSTRACT

Introduction: *Enterococci* are normal commensals in the gastrointestinal tract, oral cavity, vagina etc. They are organisms of low virulence, but are known to cause various clinical infections. *Enterococcus* is considered as an important nososcomial pathogen because of its intrinsic as well as acquired antibiotic resistance. The increasing importance of *Enterococcus* is largely due to their resistance to many antimicrobials particularly intrinsically resistant *Enterococci*, which is the cause of changing pattern of Enterococcal infection resulting in treatment failures.

Objectives: 1. Isolation, Identification and speciation of *Enterococci* from clinical specimens by conventional method. 2. Determination of Antibiogram of such isolates of *Enterococci*.

Materials and Methods: Over a period of one year, 3,197 various clinical specimens were processed and a total of 80 strains of *Enterococci* isolated. Such isolates were identified and speciated by recommended conventional tests and biochemical reactions. Their antibiotic susceptibility pattern was also determined to the common antibiotics by disc diffusion method as per the recommendations.

Results: Among the total 80 strains of *Enterococci*, 69 were *E.faecalis*, 10 were *faecium* and one was *durans* and all the isolated strains were identifiable to the species level by conventional method. Maximum numbers of isolates were from urine specimen (38, 1.97%), followed by exudates (32, 5.54%) and blood (10, 1.40%), and with an overall incidence of isolation of *Enterococci* from the above said specimens is 2.5%. Among 80 strains of *Enterococci* 35% were β hemolytic & 63.75% isolates were non hemolytic. More than 50% of the total isolates were resistant to Ampicillin (*E.faecalis* – 49.27%, *E.faecium* -90%),Penicillin(*E.faecalis*–49.27%, *E.faecium* -80%),Tetracycline(*E.faecalis*–57.97%, *E.faecium* -70%), Erythromycin(*E.faecalis* –53.62% , *E.faecium* -60%) and Gentamicin (*E.faecalis* –59.42% *E.faecium* -80%) and the most useful antibiotic was ciprofloxacin to which 61.25% (*E.faecalis* – 62.31%, *E.faecium* -50%) of the total isolates were sensitive.

Conclusion: From this study it was possible to identify all the isolates of *Enterococci* to the species level by following conventional methods. It was observed in our study that there is more drug resistance to the tested antimicrobials among *E.faecalis* isolates.

Keywords: *E.faecalis, E.faecium, E.durans, Haemolysis, Antibiogram*

INTRODUCTION

Reports on the role of *Enterococci* in infections dates back as early as 1906 from a case of endocarditis [1]. *Enterococci* have now emerged as nososcomial pathogens. In-spite of their low virulence, they are now being reported in nososcomial infections [2-4]. Their multidrug resistance limits the scope of specific antimicrobial therapy [2]. *Enterococci* need to be identified to the species level to establish the epidemiological patterns in hospitals [1]. Importance of *Enterococci* lies in their resistance to β-lactams and amino-glycosides; in particularly carrying intrinsic & acquired resistance determinants leading to life threatening infections [2-6]. Resistance to vancomycin and the emergence of vancomycin resistant *Enterococci* needs to be carefully monitored especially in tertiary care hospitals [7]. In a CDC survey of nososcomial infections, *Enterococci* contributed for 13.9% of hospital acquired UTI's, next only to the *E-coli* [8,9]. *Enterococci* are the second most common cause of nososcomial wound infections & third most common cause of nososcomial bacteremia [6,9,10].

Inanimate objects, ranging from rectal thermometer to air fluidized microsphere beds and prolonged hospital stay have led to increased colonization of *Enterococci* among the hospitalized patients [7]. The genus *Enterococci* includes many species [1,11], but commonly implicated species in human infections are *E.faecalis* & *E.faecium* [12]. Recently, there is an increase in the rate of isolation of *E.faecium* and other species from clinical specimens [1,8,9].

Transfer of Van –A gene from *Enterococci* to other Gram positive bacteria is a well known property and it makes the treatment even more difficult in nososcomial infections [9,10,13].

Hence, tertiary care hospitals have to be vigilant and need to set up laboratory procedures to isolate, identify and speciate *Enterococci* as the first step in understanding their role in nososcomial infections. CDC has accorded the same importance to multidrug resistant *Enterococci* with that of MRSA & ESBL nososcomial pathogens. The above details emphasizes the need for isolation, identification and antibiogram determination of *Enterococci* from various clinical specimens

OBJECTIVES OF THE STUDY

This study was undertaken with the following objectives

1. Isolation, Identification and speciation of *Enterococci* from clinical specimens by conventional method.

2. Determination of Antibiogram of such isolates of *Enterococci.*

MATERIALS AND METHODS

A total of 3,197 clinical specimens, received over a period of 18 months (June 2002 to November 2003) at the Microbiology Department, MMC, Mysore, India were processed, which included blood, urine and exudates like pus, ear discharge, ascitic fluid, pleural fluid, synovial fluid, CSF and corneal scrapings over a period of one year. However, among the exudates sputum, throat swab, stool and vaginal swabs were not processed & excluded from the study. Specimens were collected according to the standard recommended methods [9,14, 15,17,18].

Specimens were processed as follows-

1. **Direct Microscopy:** Smears were made from the specimens except blood and Gram's staining was done to look for pus cells & Gram positive cocci arranged in pairs, short chains or discretely.

2. **Culture:** Specimens were inoculated on to Blood agar & Mac Conkey agar as per the recommended procedures [14,15]. Such inoculated plates were incubated over night at 37^{0}C. Later the inoculated plates were examined for *Enterococci* growth as follows:

a. **Blood Agar:** Presence of small translucent colonies with α, β & no haemolysis [14].

b. **Mac Conkey agar:** tiny deep pink magenta colored colonies [14].

c. In case of urine specimen colony counting was done & only the significant counts were further processed [16]

3. Presumptive identification of *Enterococci* was done by colony smear & Gram's stain, Catalase test [14,17,18], Bile-aesculin test [14,19], Salt tolerance test [17,19-21] and Heat tolerance test [17,22,23] in which *Enterococci* are Catalase negative, hydrolyses the aesculin to aesculitin, tolerate the salt concentration of 6.5% & tolerate the temperature of 60^{0}C for 30 minutes respectively [22,23,17].

4. Speciation of *Enterococci* was done with the help of Motility, VP [24-26], Arginine hydrolysis test [20,24]. Tellurite reduction test [27,20,24] and sugar fermentation tests [18,15,28]. Results were interpreted as per the [Table/Fig-1] [28].

5. **Antibiogram:** Antibiotic susceptibility testing of the isolated strain was carried out according to the Kirby-Bauer disc diffusion method as per the standard recommendations. Susceptibility testing was quality controlled using ATCC strains of *S.aureus*, *E.faecalis*, *E.coli* & *Pseudomonas aeruginosa*. The following discs were obtained from Himedia Lab Pvt limited & tested. Zone diameters were interpreted according to the standard guidelines

a. Ampicillin - 10µgm
b. Penicillin - 10 µgm
c. Tetracycline -30 µgm
d. Erythromycin – 15 µgm
e. Ciprofloxacin- 5 µgm
f. Gentamicin – 10 µgm
g. Vancomycin – 30 µgm

RESULTS AND ANALYSIS

Total of 80 strains of *Enterococci* were isolated from a total of 3197 clinical specimens processed. Over all incidence was found to be 2.5% approximately [Table/Fig-2]. Out of the 80 strains 69 were *E.faecalis*, 10 were *faecium* and one was *durans*. Highest numbers of isolates were obtained from urine samples followed by exudates and blood. But percentage of isolation is more in exudates (5.54%) followed by urine (1.98%) & blood (1.40%) specimens [Table/Fig-2].

Maximum numbers of isolates i.e., 27 (33.75%) were from the age group 61yrs and above & least numbers of isolates i.e., 10(12.5%) were obtained from the age group between 41 to 60 yrs [Table/Fig-3]. Results of our study shows that highest numbers of isolates were *E. faecalis* (86.25%) followed by *E.faecium* (12.5%) and *E.durans* (1.25%). Maximum numbers of isolates were obtained from urine samples followed by exudates (pus, ear discharge & Ascitic fluids) and blood [Table/Fig-1].

Of the isolates, 35% were β hemolytic & 63.75% isolates were non hemolytic. Highest β hemolytic activity was found among the urinary isolates (48.48%), followed by pus (33.33%) and blood (25%) as observed from the [Table/Fig-4&5]. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Among the total isolates, resistance to Ampicillin was 55%, Penicillin-52.5%, Tetracycline-58.75%, Erythromycin-55%, Ciprofloxacin-38.75% & Gentamicin-61.25%. From the [Table/Fig-6] it is shown that the most useful antibiotics to treat *Enterococcal infections* are Ciprofloxacin to which 61.25% of the isolates were sensitive and Vancomycin to which all the isolated strains were sensitive.

Species of *Enterococci*	*VP*	*Motility*	*Arginine Hydrolysis*	*PT Reduction*	*Lactose*	*Mannitol*	*Sorbitol*	*Sorbose*	*Arabinose*	*Raffinose*	*Sucrose*
Faecalis	ND	-	+	+	+	+	+	-	-	NA	NA
Avium	-	-	-	-	NA	+	+	+	+	-	NA
Faecium	ND	-	+	-	+	+	+	-	+	NA	NA
Raffinosus	+	-	-	ND	NA	+	+	+	+	+	NA
Gallinarum	ND	+	+	+	+	+	±	-	+	NA	NA
Hirae	+	-	+	-	NA	-	-	-	NA	±	±
Casseliflavus	+	+	+	+	+	+	±	-	+	NA	NA
Durans	ND	-	+	-	NA	-	-	-	NA	-	-
Solitarius	±	-	+	ND	-	+	±	-	-	NA	NA
Mundtii	+	-	+	-	+	+	±	-	+	NA	NA
Malodaratus	-	-	-	-	NA	+	+	+	-	+	NA
Pseudoavium	+	-	-	ND	NA	+	+	+	-	-	NA

[Table/Fig-1]: Speciation was done with the help of following tests and results were interpreted as per this table
ND- Not detected; NA-Not applicable; VP- Voges-Prausker's test; PT-Potassium Tellurite Test; "+"- fermented; '-'– Negative for fermentation

Specimens	Total No.	Isolates		*E.faecalis*	*E.faecium*	*E.durans*
		Number	%			
Urine	1926	38	1.98	33	4	1
Exudates	577	32	5.54	28	4	-
a. Pus	422	27	3.39	24	3	-
b. Corneal scrapings	41	-	-	-	-	-
c. Ascitic fluid	15	2	13.3	1	1	-
d. Pleural fluid	8	1	12.5	1	-	-
e. Synovial fluid	2	-	-	-	-	-
f. CSF	28	-	-	-	-	-
g. Ear discharge	24	2	8.3	2	-	-
Blood	694	10	1.44	8	2	0
Total isolates	3197	80	2.5	69	10	1

[Table/Fig-2]: Species distribution among different individual specimens

Age groups (years)	ables		Exudates	Urine	Blood*	Total	Percentage
	M (%)	F (%)					
0-20	11 (44)	14(56)	5	11	9	25	31.25
21-40	7 (38.9)	11(61.1)10	10	7	1	18	22.5
41-60	4(40)	6(60)7	7	3	0	10	12.5
≥61	23(85.2)	4(14.9)	10	17	0	27	33.7
TOTAL	45(56.2)	35(43.7)	32	38	10	80	

[Table/Fig-3]: Age and sex distribution of isolates
*Blood isolates: 5 out of 9 isolates are from sick babies less than 3 months of age

Among the *E.faecalis* isolates more than 50% of the isolates were resistant to Tetracycline, Erythromycin and Gentamicin. 50.72% (35) isolates were sensitive to Ampicillin and 62.3% (43) isolates were sensitive to Ciprofloxacin. All the isolates were susceptible to vancomycin – [Table/Fig-5].

Among the *E.faecium* isolates 90% (9) isolates were resistant to Ampicillin and 50% (5) isolates were resistant to Ciprofloxacin. All the isolates were sensitive to vancomycin – [Table/Fig-5].

Specimen	Isolates (No)	Non hemolytic		β hemolytic		α hemolytic
		No	%	No	%	
Pus	24	16	66.66	8	33.33	-
Body fluids	2	2	-	-	-	-
Ear discharge	2	2	-	-	-	-
Blood	8	6	75	2	25	-
Urine	33	17	51.51	16	48.48	-
Total	69	43	62.31	26	37.69	

[Table/Fig-4]: Hemolytic activity of *E.faecalis* in individual specimens

Specimen	Isolates (No)	α hemolytic	β hemolytic	Non hemolytic
Pus	3	-	1	2
Body fluids	1	-	-	1
Urine	4	-	1	3
Blood	2	-	-	2
Total	10	-	2 (20%)	8(80%)

[Table/Fig-5]: Hemolytic activity of *E.faecium* isolates

Antibiotics	Disc strength	Total isolates				*E.faecalis*				*E.faecium*			
		R	(%)	S	(%)	R	(%)	S	(%)	R	(%)	S	(%)
Ampicillin	10µg	44	55	36	45	34	49.27	35	50.72	9	90	1	10
Penicillin	10µg	42	52.5	38	47.5	34	49.27	35	50.72	8	80	2	20
Tetracycline	30 µg	47	58.75	33	41.25	40	57.97	29	42.02	7	70	3	30
Erythromycin	15 µg	44	55	36	45	37	53.62	32	46.37	6	60	4	40
Ciprofloxacin	5 µg	31	38.75	49	61.25	26	37.68	43	62.31	5	50	5	50
Gentamicin	10 µg	49	61.25	31	38.75	41	59.42	28	40.57	8	80	2	20
Vancomycin	30 µg	-	-	80	100	-	-	69	100	-	-	10	100

[Table/Fig-6]: Antibiogram of total isolates, *E.faecalis* & *E.faecium* spp.
S-sensitive, R-resistance

DISCUSSION

Enterococci have been considered as the normal flora of the intestinal tract, oral cavity, vagina, etc. They have been associated with many types of infections especially as nososcomial pathogens. Therefore, it is important to know the patterns of infections caused by *Enterococci* and their Antibiogram. This study investigated the conventional method of isolation, identification and antimicrobial susceptibility pattern of *Enterococci* from various clinical samples. Battery of tests used in the present study identified all the isolates to the species level. Conventional tests proposed by R.R.Facklam and Collins were thus successfully used for the speciation of *Enterococci* [20].

In this study highest number of isolates were obtained from urine specimen but percentage of isolation is more among the exudates which is similar to many other studies [29,30,31] [Table/Fig-1].

Maximum numbers of isolates were obtained from the age group ≥ 61 yrs with more predominance in males compared to females [Table/Fig-3] which is similar to other studies [32,33]. But in another study maximum isolates were obtained from age group between 21 to 40 yrs with female predominance [31].

As comparable with many studies the majority of the isolates were *E.faecalis* followed by *E.faecium* and one isolate was *E.durans* –[Table/Fig-2].

Hemolytic activity gives additional information on the pathogenic role of *Enterococci* and in this study, of the total isolates 63.75% were non hemolytic and 35% were β hemolytic. In a study only 5% were β hemolytic and 80% percent were non hemolytic [34]. Majority of the isolates which are β hemolytic in this study were from urine specimens followed by exudates.

Although the prevalence of β lactamase producing strains is low, all isolated strains should be tested for β lactamase production [35]. In the present study, such detection was not

done, however the penicillin resistance was found to be 49.27 % in *E.faecalis* and 80% in *E.faecium*.

In the present study, resistance to Gentamicin, which is commonly used in conjunction with penicillin, was found to be more than 50% among both *E.faecalis* & *E.faecium* isolates, which is comparable with the other studies [31]. This type of resistance leaves the clinicians with limited choice of using newer drugs like Linezolid, Tigecycline etc [31].

In this study it was noted that, *E.faecium* showed more resistance to the tested drugs compared to the *E.faecalis* which correlates with the other studies [36,37]. Resistance against Tetracycline, Erythromycin and Gentamicin among both types of isolated species in the present study was found to be more than 50%, which is comparable with other studies [33].

CONCLUSION

In this study the most common isolate was *E.faecium* followed by *E.faecium*. But recently there is change in the species isolated from the clinical specimens i.e., more commonly the *faecium* species are isolated. This suggests that there is change in the pattern of infections caused by the *Enterococci*. Vancomycin resistant strains have been increasingly reported from all over the world. However in the present study no such strains were isolated.

There is increased literature evidence showing that multi drug resistance is prevalent among the *Enterococci* around the world. This suggests that there should be continuous or periodic surveillance among the dynamics of the infections caused by the *Enterococci* at least in the hospitals at all levels, which will help to treat the patients effectively especially among the hospitalized patients.

Prevention and control of spread of multi drug resistant *Enterococcal infections* in the hospital require a co-ordinated effort between the various departments and this can only be achieved by educating the hospital staff, vigilant use of antimicrobials, early detection and reporting by laboratories and immediate implementation of appropriate infection control measures.

REFERENCES

[1] Murray E Barbara. Life and Times of *Enterococcus*. *Clinical Microbiological Reviews*. 1990. 3(1):46-65.

[2] Vandamme P, Vercauteran E, Lammens C, Pansart N, Ievan M, Pot B, et al. Survey of Enterococcal Susceptibility Pattern in Belgium. *J Clin Microbial.* 1996: 34:2572-76.

[3] Eliopoulos GM. Increasing problems in the therapy of *Enterococcal infections*. *Eur J Clin Microbial Infect Dis.* 1993; 12; 409-12.

[4] Willey B, McGeer AJ, Ostrowski MA, Krieswirth BN, Low DE, The use of molecular typing technique in the epidemiological investigation of resistant *Enterococci* Infect. *Control Hosp. Epidemiol* 1994; 15: 548-56.

[5] Hall LMC, Recent advances in understanding the epidemiology of *Enterococci*. *Rev Med Microbiol.* 19936; 4: 192-97.

[6] Moellering RC. Emergence of *Enterococci* as Significant Pathogen. *Clin Infect Dis*. 1992; 14:1173-78.

[7] Cetinkaya Yesim, Falk Pamela and Mayhall CGlen. Vancomycin Resistant *Enterococci*. *Clinical Microbiology Reviews*. 2000: 13 (4): 686-707.

[8] Desai PJ, Pandith D, Mathur M, Gogate A.Prevalence, Identification and distribution of various species of *Enterococci* isolated from clinical samples with special reference to urinary tract infections in catheterized patients. *Indian J Med Microbial.* 2001; 19:132-37.

[9] Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Isolation and Identification *Streptococci* like organisms" Koneman's Colour Atlas and Text Book of Diagnostic Microbiology, 6th ed; *Baltimore Lippincott William and Wilkins.* 2006:726-33.

[10] Felmingham D, Wilson APR, Quintana AI, Grunberg RN. *Enterococcus* species in urinary tract infection. *Clin Infect Dis.* 1992;15;295-301.

[11] Jett BD, Huycke MM, Gilmore MS. Virulence of *Enterococci Clinical Microbiology Reviews*. 1994;7(4):462-78.

[12] Morrison D, Woodford N, Cookson B. *Enterococci* as emerging pathogens of humans. *Journal of Applied Microbiology Symposium Suppl*. 1997:83:89s-99s.

[13] Noble WC, Virani Z, Cree RGA, Co-transfer of Vancomycin and other resistance genes from *Enterococcus* Faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett. 1992;93:195-92.

[14] Koneman EW, Allen SD, Janda WM et al. Introduction to microbiology Part 1. Chapter 2 in clour Atlas and Text Book of Diagnostic Microbiology, 5th Ed Lippincott William and Wilkins, 1997:76-78 and 1307-12.

[15] Forbes BA, Sahm DF, Weissfeld AS.Overview of Bacterial Identification Methods and Strategies. Bailey and Scott's Diagnostic Microbiology, 12th Ed, Mosby, 2007; 216-47.

[16] Popa R.I, Gray LA, Kallmes DF, .Urinary Tract Infections in the Potential Vertebroplasty Patient: Incidence, Significance and Management. *Am J Neuroradiol*. 2009; 30:227-31.

[17] Collee JG, Marr W "Specimen collection, culture containers and media" Chapter 5 in Mackie and McCartney Practical Medical Microbiology, 14th Ed, Churchill Livingstone, 1996:95-112.

[18] Ross PW .*Streptococci* and *Enterococci*. Mackie and McCartney Practical Medical Microbiology, 14th Ed Elsievier 2006;268-69.

[19] Facklam RR. Recognition of Group D Streptococcal species of human origin by biochemical and physiological tests. *J Appl Microbiol*. 1972;23(6):1131-39.

[20] Facklam RR, Collins MD. Identification of *Enterococci* species isolated from human infection by a conventional test scheme. *Journal of Clinical Microbiology*. 1989; 27(4):731-34.

[21] Hussain SM Quadri, Charles W Nicholas, Quadri SGM. Rapid Sodium chloride tolerance test for presumptive identification of *Enterococci*. *Journal of Clinical Microbiology.* 1978; 7(2):238-39.

[22] Facklam RR, Moody MD. Presumptive Identification of Group D *Streptococci*: Bile-esculin test. *Journal of Appl Microbiology.* 1970; 20(2):245-50.

[23] James M Sherman. The *Streptococci*. *Bacteriology Reviews.* 1937; 1:3-97.

[24] Rouff LK, Spargo DJ, Ferraro JM. Species Identification of *Enterococci* Isolates from clinical specimens. *J Clin Microbiol.* 1990; 28:435-37.

[25] Koneman EW, Allen SD, Janda WM et al. Gram Positive Cocci Part-II. Chapter 2 in color Atlas and Text Book of Diagnostic Microbiology, 5th Ed Lippincott William and Wilkins. 1997:616-18.

[26] Fertally SS, Facklam R "Comparison of Physiological tests used to identify non beta haemolytic Aerococci, *Enterococci* and *Streptococci*. *J. Clin.Microbiol*. 1987; 25(10):1845-50.

[27] Deibel RH. Group D *Streptococci. Bacteriol Rev*. 1964;28:330-66.
[28] Udo EE, Naura Al-Sweith. Species prevalence and anti bacterial resistance of *Enterococci* isolated in Kuwait hospitals. *J. Med. Microbiol.* 2003; 52:163-68.
[29] Narayan Shrihari, Kumudini TS, Kardesai SG, Metgud SC. Speciation of entrococcal isolates and antibiotic susceptibility test including high level aminoglycoside resistance and minimum inhibitory concentration for vancomycin. *Int J Biol Med Res.* 2011; 2(4):865-69.
[30] Bhakare Ujjwala, Thombare Vilas, Nagdeo Neena. Identification of *Enterococcus* species isolated from clinical specimens and detection of vancomycin resistant *Enterococci*. *Int J Pharm Bio Sci.* 2014; 5(2):(B)847-52.
[31] Preeti S, Raman Mehta, Nirwan PS, Meeta Sharma, Dahiya SS. Prevelance and antimicrobial susceptibility of *Enterococcus* species isolated from different clinical samples in a tertiary care hospital of North India. *National J Med Res*. 2013; 3(4): 389-91.
[32] Saroj Golia, Nirmala AR, Asha S Kamath B. Isolation and speciation of *Enterococci* from various clinical samples and their antimicrobial susceptibility pattern with special reference to high level aminoglycoside resistance. *Int J Med Res Health Sci.* 2014; 3(3):526-29.
[33] Parameshwarappa J, Basavaraj VP, Basavaraj CM. Isolation, Identification and Antibiogram of *Enterococci* isolated from patients with urinary tract infection. *Ann Afr Med.* 2013; 12(3):176-81.
[34] Gulati V, Aggarwal A, Khanna S, Narang V K. Biochemical speciation of *Enterococci* causing human infections. *Indian J Med Sci.* 1997;51:310-12.
[35] Shinde RS, Koppikar GV, Oommen S. Charecterization and antimicrobial susceptibility pattern of clinical isolates of *Enterococci* at a tertiary hospital in Mumbai. *India Ann Trop Med Public Health.* 2012;5:85-88.
[36] Suresh K, Saipriya B, Vishwanath G. Isolation speciation and determination of High level Aminoglycoside Resistance of *Enterococci* among hospitalized patients in Davanagere. *National Journal of Laboratory Medicine.* 2014; 2:12-15.
[37] Agarwa Jyothsna I, Kalyan Rajkumar, Mastan Singh. High level aminoglycoside resistance and β lactamse production in *Enterococci* at a tertiary hospital in India. *Jpn J Infect Dis*. 2009; 62:158-59.

AUTHOR(S):

1. Dr. Shanmukhappa
2. Dr. D Venkatesha
3. Dr. Ajantha G S
4. Dr. Amrutha Kumari B
5. Dr. Mallikarjun Koppad

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Mandya Institute of Medical Sciences Mandya, India.
2. Professor & HOD, Department of Microbiology, Mysore Medical College, Mysore, India.
3. Professor, Department of Microbiology, SDMC, Dharwad, India.
4. Associate Professor, Department of Microbiology, Mysore Medical College, Mysore, India.
5. Assistant Professor, Department of Microbiology, Institute of Medical Sciences, Shimoga, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Shanmukhappa
Associate Professor, Department of Microbiology, Mandya Institute of Medical Sciences, Mandya,
Karnataka-571401, India.
E-mail : shaansv@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Apr 01, 2015**