

Original Research Article

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## Evaluation of Sterile Body Fluids Infection and its Antimicrobial Susceptibility Pattern in a Tertiary Care Centre in South India

Sanjana Upadhyay<sup>ID</sup>\* and Anil Kumar Bilolikar

Department of Microbiology, Krishna Institute of Medical Sciences, Minister Road,  
Secunderabad - 500003, Telangana, India

\*Corresponding author

### ABSTRACT

#### Keywords

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The infection of sterile body fluids is potentially significant as growth of even one colony of a pathogenic microorganism can lead to life threatening consequences. Less number of pathogens and prior empirical antibiotic therapy in patients result in very fewer positive cultures. Hence the study was conducted to isolate the causative organisms of sterile body fluid infections and determine their antimicrobial susceptibility pattern. The body fluids received in the department of Microbiology KIMS Secunderabad were processed in BacT/ALERT 3D by inoculating them in FA Plus bottles under aseptic conditions for 5 days. 2190 samples studied out of which 1110 (50.68%) were cerebrospinal fluid, 498(22.73%) were pleural fluids, 456 (20.82%) were ascitic fluids, and 126(5.75%) were synovial fluid. The predominantly isolated bacteria were *Klebsiella pneumoniae* followed by *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus spp.*, *Viridans streptococci*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Acinetobacter spp.*, *Serratia*, *Enterobacter cloacae*, *Streptococcus pneumonia* and other Gram negative bacteria. Gram negative isolates were 100% sensitive to Colistin followed by Tigecycline (85%), Carbapenems (80%), Cefoperazone sulbactam (62%) and Cefepime (62%). *Staphylococcus* isolates were maximum sensitive to Vancomycin, Linezolid and Tigecycline. Among GNBs (*enterobacterales*) high resistance was observed to Ciprofloxacin(15.3%) and Amoxicillin clavulanic acid (30.7%). The current shift is an ever-increasing speed of antibiotic resistance in both gram negative and gram-positive isolates. It calls for a strict and organised surveillance and monitoring in hospitals, so the physicians can immediately acquire latest details on prevalence and antimicrobial susceptibility pattern which helps in shaping their clinical therapeutic judgement.

### Introduction

The infection of sterile body fluids is potentially significant as growth of single colony of a microorganism can lead to life threatening

consequences. (Bailey and Scott's, 2017). The pathogen, usually bacteria may breach the protective anatomical and physiological barrier due to the poor host's immune response and infiltrate the body fluids resulting in sequela which can be fatal. (Rouf

and Nazir, 2019; Sharma *et al.*, 2017). The isolation, identification and antimicrobial susceptibility profile of the causative agent can be a crucial factor for patient treatment. (Daur *et al.*, 2006; Hughes *et al.*, 2001)

The common pathogenic bacteria of concern are *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas*, *Acinetobacter spp.*, *Staphylococcus aureus* and *Enterococcus spp.*

Nevertheless, fewer positive cultures are noticed attributing to several factors-less number of pathogens, prior empirical antibiotic therapy, fastidious organisms, and due to use of conventional culture methods which requires large quantity of fluid samples relatively for inoculation leading to delayed result as well as contamination.

Alternatively, automated blood culture system like BacT/ALERT 3D uses enriched media such as tryptic soy broth and/ or brain heart infusion broth for greater yield added with adsorbent polymeric resin beads which neutralize the antimicrobials present in the body fluids.

Also these have continuous monitoring for microbial growth once in every 10 minutes by the instrument. Hence these samples are inoculated in such media broth and loaded into the system for early and rapid identification of microorganisms.

Since the study on sterile body fluid infection and its antimicrobial susceptibility pattern pertaining to our geographical area is scant; collecting, analysing and interpreting such data can help create local antibiogram which in turn guide the clinicians to initiate more specific patient treatment, with less adverse effects and reduced lengths of hospital stay. (Wiest *et al.*, 2012; Van de Beek *et al.*, 2012)

Therefore, the current study was conducted to identify the causative organisms of sterile body fluid infections and their antimicrobial susceptibility pattern in patients attending our tertiary care hospital and recognize any shift in the trend.

## **Materials and Methods**

This was a prospective study on sterile body fluids received over a period of 2 years (September 2019 to August 2021) in the department of Microbiology, KIMS Secunderabad. It was approved by Ethical and Scientific committee of the institute.

The body fluids like Cerebrospinal, Pleural, Ascitic and Synovial fluids were included in the study and collected with utmost sterile precautions. The quantity of body fluids available for culture was in the range of 0.5 to 5.0 ml. These were processed in BacT/ALERT 3D by inoculating them in FA Plus bottles within 2 hours of collection under aseptic conditions for 5 days.

## **Inclusion Criteria**

CSF, Pleural, Ascitic and Synovial fluids from all the patients with suspected body fluid infections, admitted in KIMS hospital Secunderabad, irrespective of age and sex were included.

## **Exclusion Criteria**

1) Pericardial fluid, 2) Blood samples 3) Patients with history of antibiotics within the last 2 weeks 4) Old and contaminated samples

When the inoculated BacT/ALERT FA Plus culture bottle flagged positive, it was checked for bacterial growth curve and change in color of the gas-permeable sensor installed in the bottom of the bottle to yellow, following which they were unloaded and subcultured on blood agar (BA), MacConkey agar (MA), chocolate agar (CA) and fluid thioglycollate medium (FTM). The blood agar, chocolate agar and FTM were incubated in a 5-10% CO<sub>2</sub> enriched atmosphere at 35-37°C for 24-48 hrs and checked for growth after overnight incubation. The MacConkey agar plate was incubated aerobically at 35-37°C overnight.

If culture showed growth, the identification and antimicrobial susceptibility testing (AST) of bacteria

was done by using VITEK 2 compact (BIOMERIEUX); the susceptibility profile was interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations. (CLSI, 2019)

The antibiotics used for Gram positive organisms included, penicillin (P), oxacillin, fluoroquinolones (FQs) - ciprofloxacin and levofloxacin, linezolid (LNZ), vancomycin (VA), tetracycline (TE), tigecycline (TGC), clindamycin (CM), gentamicin (GM), ceftriaxone (CRO) and cotrimoxazole (SXT).

The antibiotics used for lactose fermenting Gram negative bacilli (Enterobacterales) included amoxicillin-clavulanic acid (AMC), ceftriaxone (CRO), cefepime (CEF), cefoperazone-sulbactam (SFP), carbapenems (CRS)- ertapenem, imipenem, meropenem, aminoglycoside (AMG)- amikacin, gentamicin, ciprofloxacin (CIP), tigecycline (TGC), colistin (CS) and cotrimoxazole (SXT).

The antibiotics used for Non fermenting Gram negative bacilli (NFGNB) included ticarcillin-clavulanic acid (TCC), ceftazidime (CAZ), cefepime (CEF), cefoperazone-sulbactam (SFP), carbapenems (CRS) -doripenem, imipenem, meropenem, aminoglycoside (AMG) - amikacin, gentamicin, ciprofloxacin (CIP), piperacillintazobactam (TZP), tigecycline (TGC), colistin (CS), cotrimoxazole (SXT).

## **Results and Discussion**

Out of 2190 cultured sterile body fluid specimens, 1426 and 764 fluids were from the males and females respectively. Among these, CSF samples (1110) made up 50.68%, pleural fluid samples (498) 22.74%, peritoneal fluid (456) 20.82%, and synovial fluid (126) 5.76% as shown in Fig.1.

264 samples showed positive culture with an isolation rate of 12.05%, of which Gram-negative organisms constituted 56.06%, gram positive organisms 40.91% and *Candida* 3.03% as shown in Fig. 2. The rest 1926 (87.95%) were reported as no bacterial growth observed.

Amidst the Gram negative organisms, the most common was *Klebsiella pneumonia* (19.7%) and in the Gram positive organisms, the most common pathogen was *Staphylococcus aureus* (18.9%) as shown in Fig. 3.

The rate of isolation of various organisms sample wise is shown in Fig 4.

The AST profile of gram positive organisms, gram negative *Enterobacterales* and non fermenters are shown in tables 1, 2, 3 respectively

The drug resistance in *Staphylococcus aureus*, *Enterococcus*, *Enterobacterales* and non fermenters are shown in fig 5, 6 respectively.

Sterile body fluids infection is now prevailing in most of the developing as well as developed countries. In the present study, the prevalence of infection was 12.05% which was nearly similar to the findings of other studies done in India, 10.81% (Rouf and Nazir, 2019), 14.78% (Vishalakshi *et al.*, 2016), 14.8% (Kasana *et al.*, 2015), 14.41% (Deb *et al.*, 2014) and 14.1% (Teklehymanot *et al.*, 2017).

In contrast, some studies showed a higher prevalence of 30% (Sharma *et al.*, 2017), 31% (Sujatha *et al.*, 2015) and 22% (Harshika *et al.*, 2018) as shown in Table 4. This might be due to over diagnosing, presence of anaerobic fastidious organisms, prior exposure to antibiotics and emergence of non-infectious conditions like malignancy. (Teklehymanot *et al.*, 2017; Sujatha *et al.*, 2015)

From the positive cultures, CSF accounted for 50.68 %, pleural fluid 22.74%, ascitic fluid 20.82%, and synovial fluid 5.76%. Similar observation was seen in study by Rouf and Nazir (2019).

Here, Gram-negative isolates had a culture positivity rate of 56.06% as compared to gram positive isolates (40.91%). This was also observed in other studies done by Rouf and Nazir (2019); Sharma *et al.*, (2017); Sujatha *et al.*, (2015) and Harshika *et al.*,

(2018), whereas studies by Vishalakshmi *et al.*, (2016); Bourbeau *et al.*, (1998) and Yoon *et al.*, (2010) showed higher isolation rate of gram positive organisms. This could be due to inclusion of body fluid samples other than CSF, ascitic fluid, like pleural fluid, synovial fluid, dialysis fluid, pericardial fluid which accounted for majority of sample size. The discrepancy in distribution of samples can explain the variance in the type of commonly isolated pathogens.

Amongst the Gram negative organisms, the most common were *K.pneumoniae* (19.7%), *E. coli* (18.9%) followed by *P. aeruginosa* (4.5%), *E. cloacae* (2.3%), *Acinetobacter spp* (2.3%), *Serratia spp.* (2.3%), *Salmonella Typhi* (1.5%) and other GNBs. From Gram positive isolates, the most common pathogen was *S. aureus* (18.9%) followed by *Enterococcus spp.* (9.1%), *Viridans streptococci* (5.3%), *S. pyogenes* (3%), *CONS* (3%) and *S. pneumoniae* (1.5%).

This finding was similar to studies done by Rouf and Nazir (2019); Deb *et al.*, (2014); Sujatha *et al.*, (2015); Harshika *et al.*, (2018) and Lakshmi (2001) where predominantly *Enterobacteriales* were isolated. However, *Acinetobacter spp.* was observed as one of the common isolate in the study performed by Sharma *et al.*, (2017).

Maximum growth was observed in ascitic fluid followed by CSF, pleural fluid and synovial fluid. In ascitic fluid, Gram negative isolates were more commonly detected; *E. coli* was the most common followed by *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *Salmonella Typhi* and other GNBs which was similar to the study of Rouf and Nazir (2019); Sharma *et al.*, (2017); Sujatha *et al.*, (2015); Arroyo *et al.*, (2000) and Chawla *et al.*, (2015). In the study by Harshika *et al.*, (2018), NFGNB was the most common isolate from ascitic fluid. Among Gram positive bacteria, *Enterococcus* was the most common organism followed by *S. aureus* and *Viridans streptococci* isolated from ascitic fluid,

whereas in other studies *S. aureus*, *CONS* and *Enterococcus* were the common organisms isolated (Rouf and Nazir, 2019 and Harshika *et al.*, 2018).

In CSF also, Gram negative isolates were the most common compared to Gram positive isolates- *K. pneumoniae* followed by *E. coli*, *Serratia spp.*, *E. cloacae*, *Acinetobacter spp.* and other GNBs; *S. aureus* followed by *S. pyogenes*, *Enterococcus*, *Viridans streptococci* and *CONS*. Similar findings were seen in other studies. (Sujatha *et al.*, 2015; Harshika *et al.*, 2018; Mani *et al.*, 2007 and Tang *et al.*, 1999) In contrast, Rouf and Nazir (2019) observed that NFGNB like *Acinetobacter spp.* and *Pseudomonas aeruginosa* were the common bacterial isolates from CSF.

In our study, Gram negative and Gram positive organisms were isolated in same number from pleural fluid- *K. pneumoniae* followed by *P. aeruginosa*, *E. coli*, *Acinetobacter spp.*, and other GNBs; *S.aureus* followed by *Viridans streptococci*, *S. pneumoniae* and *CONS*.

However other studies reported more gram negative organisms such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter spp.* (Rouf and Nazir, 2019; Sharma *et al.*, 2017; Kasana *et al.*, 2015; Deb *et al.*, 2014 and Harshika *et al.*, 2018), whereas Vishalakshmi *et al.*, (2016) and Sujatha *et al.*, (2015) observed more Gram positive organisms such as *S. aureus* and *S. pneumoniae* from pleural fluid. It was observed that the detection of Gram Negative bacteria from pleural fluid relates to intense antimicrobial therapy with a poor outcome.

In case of synovial fluid, Gram positive organisms were the most common isolates- *S. aureus* followed by *CONS*. Among GNBs, *E. coli* and *S. Typhi* were isolated. Some other studies like Rouf and Nazir (2019) found *S. aureus*, *Pseudomonas spp.*, *Enterobacter spp.* and *Serratia marcescens* from the synovial fluid whereas Sharma *et al.*, (2017) isolated *S. aureus*, *Klebsiella spp.*, and *Enterococcus spp.*

**Table.1** Antimicrobial susceptibility pattern of gram-positive bacteria (n=108)

Bacterial isolate	P	Oxacillin	FQ	LNZ	VA	TE	TGC	CM	GM	CRO	SXT
<i>S. aureus</i> (50)	10 (20%)	30 (60%)	20(40 )	50 (100%)	50 (100%)	50 (100%)	50 (100%)	48 (96%)	48 (96%)	50 (100%)	50 (100%)
<i>Enterococcus</i> (24)	4 (16.66 )	0	4 (16.66 )	24 (100%)	14 (58.33%)	8 (33.33 )	22 (91.66 )	0	HLG 10 (41.66%)	0	0
<b>Viridans streptococci</b> (14)	6 (42.86 )	0	12 (85.71 )	14 (100%)	12 (85.71%)	6 (42.86 )	12 (85.71 )	12 (85.71 )	12 (85.71%)	8 (57.14 )	12 (85.71 )
<i>S. pyogenes</i> (8)	8 (100%)	0	4 (50%)	8 (100%)	8 (100%)	4 (50%)	8 (100%)	4 (50%)	8 (100%)	8 (100%)	8 (100%)
<i>S.pneumoniae</i> (4)	4 (100%)	0	4 (100%)	4 (100%)	4 (100%)	0	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
<b>CoNS(8)</b>	0	6 (75%)	4 (50%)	8 (100%)	8 (100%)	6 (75%)	8 (100%)	8 (100%)	8 (100%)	0	8 (100%)

**Table.2** Antimicrobial susceptibility pattern of Enterobacterales (n=124)

Bacterial isolate	AMC	CRO	CEF	SFP	CRS	AMG	CIP	TGC	CS	SXT
<i>K.pneumoniae</i> (52)	16 (30.77 )	28 (53.85%)	32 (61.54%)	32 (61.54%)	42 (80.77%)	34 (65.38 )	22 (42.3%)	44 (84.61 )	52 (100%)	44 (84.61 )
<i>E.coli</i> (50)	18 (36%)	26 (52%)	26 (52%)	22 (44%)	42 (84%)	42 (84%)	8 (16%)	50 (100%)	50 (100%)	46 (92%)
<i>Serratia spp</i> (6)	0	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33 )	6 (100%)	0	0	6 (100%)
<i>E. cloacae</i> (6)	0	4 (66.66%)	4 (66.66%)	4 (66.66%)	4 (66.66%)	6 (100%)	4(66.66 )	6(100%)	6(100 )	6(100%)
<i>Salmonella</i> (4)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	0	2 (50%)	4 (100%)	4 (100%)	4 (100%)
<i>Pantoea</i> (4)	4 (100%)	2 (50%)	2 (50%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
<i>Raoultella</i> (2)	0	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)

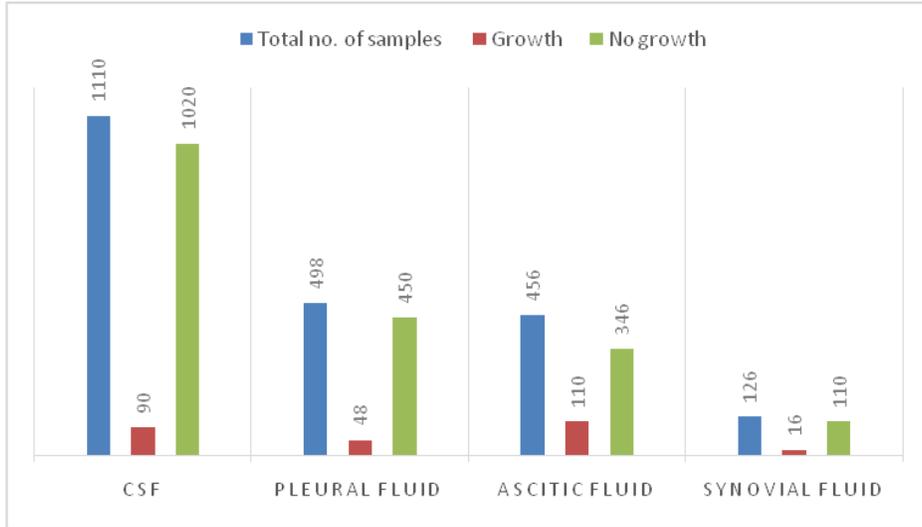
**Table.3** Antimicrobial susceptibility pattern of Non- fermenting gram-negative bacteria (n=24)

Bacterial isolate	TCC	CAZ	CEF	SFP	CRS	AMG	CIP	TZP	CS	TGC	SXT
<i>P.aeruginosa</i> (12)	0	0	0	6 (50%)	8 (66.66%)	8 (66.66%)	4 (33.33%)	0	12 (100%)	0	0
<i>Acinetobacter</i> spp (6)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	2 (33.33%)	6 (100%)	4 (66.66%)	6 (100%)
<i>S. paucimobilis</i> (2)	0	0	0	0	0	2(100%)	2(100%)	2(100%)	0	0	2(100%)
<i>B.cepacia</i> (2)	2(100%)	0	0	0	2(100%)	0	0		0	0	0
<i>Aeromonas</i> (2)	0	0	0	0	0	2(100%)	2(100%)	2(100%)	0	2(100%)	2(100%)

**Table.4** Showing comparison of prevalence of sterile body fluid infections in the present study with other studies

Sterile body fluids	Present Study (KIMS)	Rouf M <i>et al</i> 2-2019	Harshika YK <i>et al</i> 14- 2018	Sharma R <i>et al</i> 3-2017	Vishalakshi B <i>et al</i> 9-2016
Prevalence of culture positive isolates	12.05%	10.81%	22%	30%	14.78%
CSF	34.09%	34.09%	16.20%	0	0
Ascitic fluid	41.67%	23.86%	39.44%	67.21%	11.76%
Pleural fluid	18.18%	15.90%	41.55%	18.03%	5.88%
Synovial fluid	6.06%	5.68%	0	13.11%	11.76%

**Fig.1** Showing sequence of growth observed in various samples



**Fig.2** Showing division of Gram positive and Gram negative organism

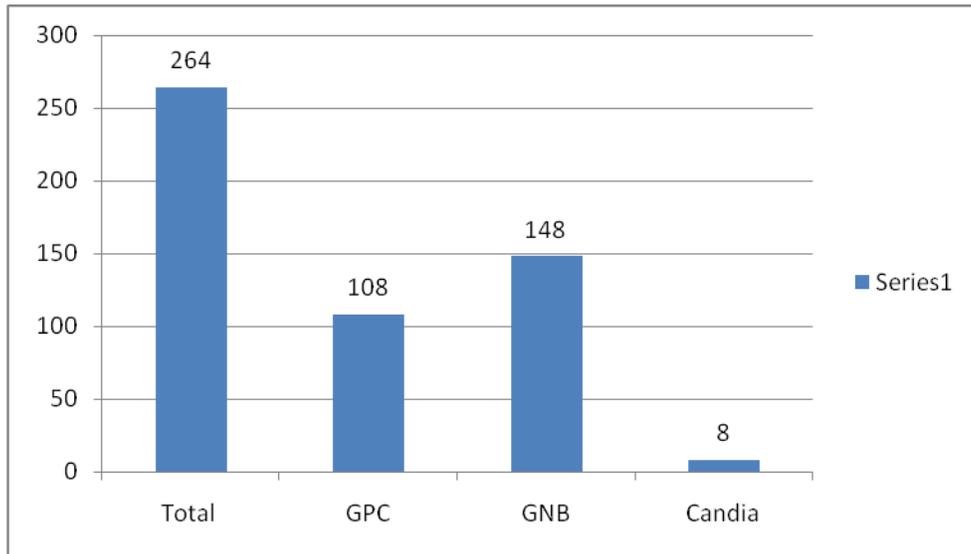


Fig.3 Showing Rate of isolation of various organisms

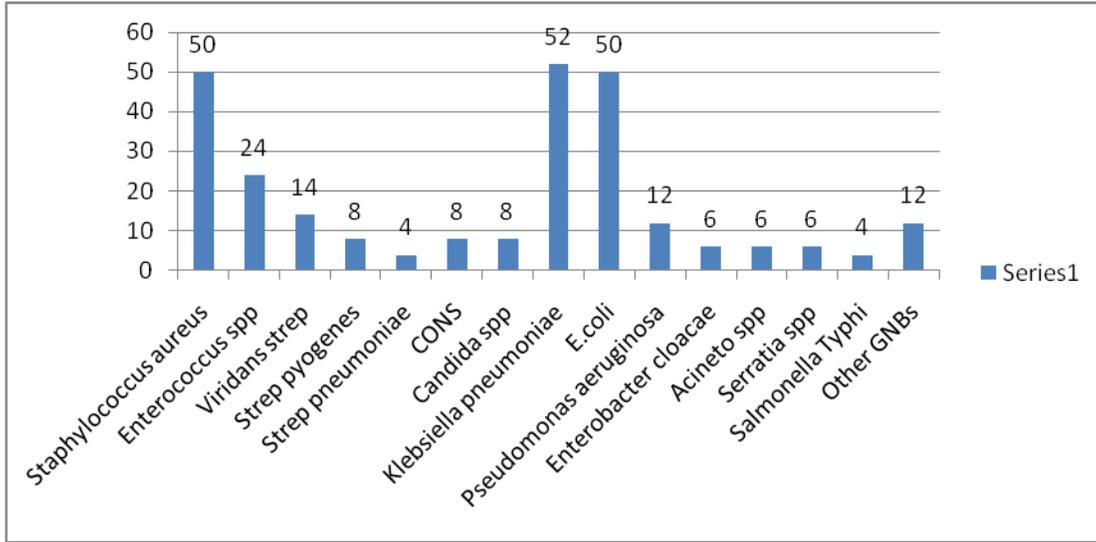


Fig.4 Showing Rate of isolation of various organisms sample wise

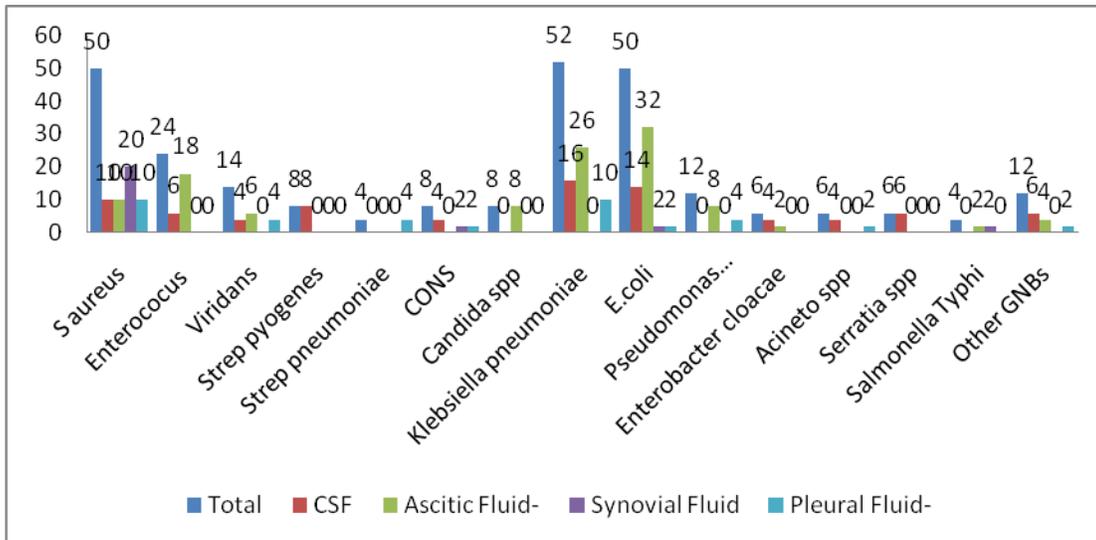


Fig.5 Showing drug resistance in *Staphylococcus aureus* n=25 & *Enterococcus* species n=12

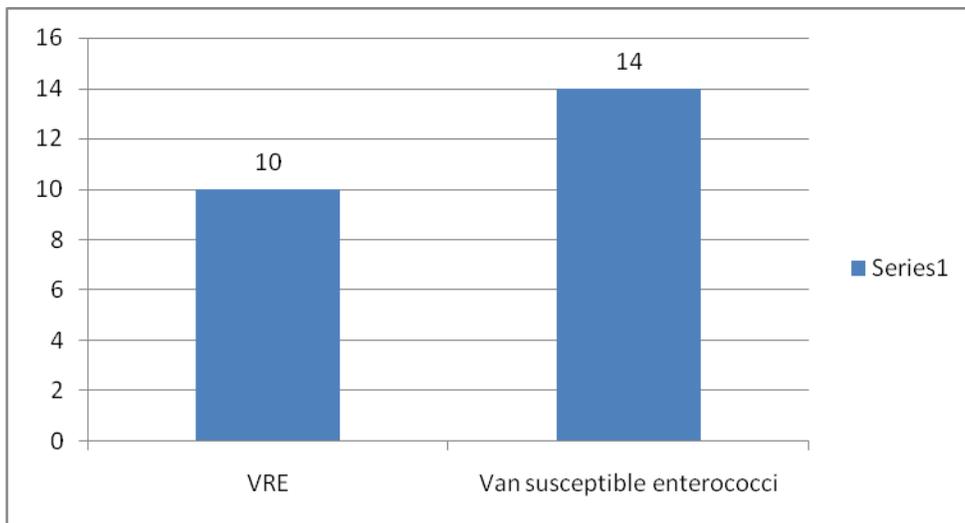
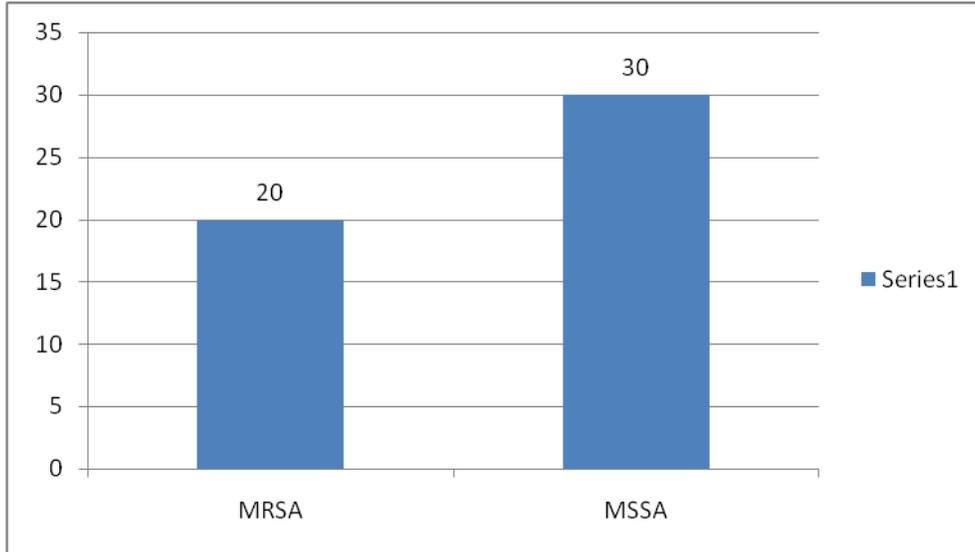
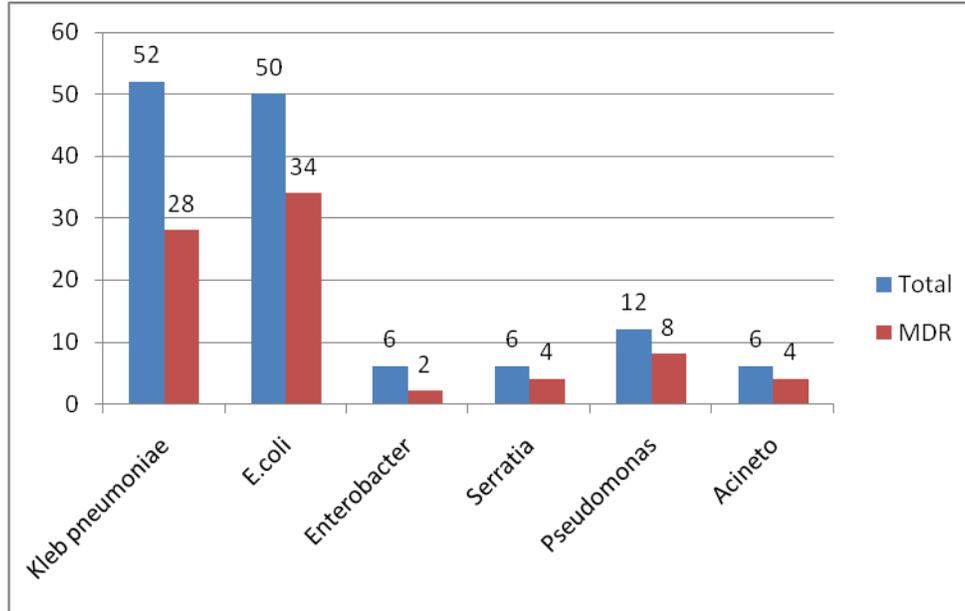


Fig.6 Showing MDR in Enterobacterales and NFGNB



In the current study, the efficacious antibiotic against *Enterobacterales* were colistin, tigecycline, cotrimoxazole followed by aminoglycosides and carbapenems. *K. pneumoniae*, *E.coli* and *Serratia spp.* were found to be resistant mostly to fluoroquinolones and cephalosporins. These finding was similar to other studies. (Rouf and Nazir, 2019; Sharma *et al.*, 2017; Harshika *et al.*, 2018 and Barai *et al.*, 2010)

Amongst the NFGNB, the crucial drug were colistin, cotrimoxazole, aminoglycosides and carbapenems, which was correlating with the findings of Tullu *et al.*, (1998) and Rouf and Nazir (2019) which also showed 100% sensitivity to colistin. *P. aeruginosa* and *Acinetobacter spp.* were the most resistant to cephalosporins, ticarcillin-clavulanic acid and piperacillin-tazobactam. In contrast, Sharma *et al.*, (2017) and Sujatha *et al.*, (2015) showed that *Pseudomonas* isolates were highly sensitive to piperacillin-tazobactam. *P. aeruginosa* and *Acinetobacter spp.* Infections have become a chief health challenge, particularly in ICUs and high risk areas. (Acosta *et al.*, 2011)

In the Gram positive organisms, 100% sensitivity to linezolid was observed followed by vancomycin,

tigecycline and cotrimoxazole. Other studies also showed maximum sensitivity to vancomycin and linezolid (Rouf and Nazir, 2019; Sharma *et al.*, 2017; Vishalakshi *et al.*, 2016; Sujatha *et al.*, 2015 and Harshika *et al.*, 2018). In our study 40% of *S. aureus* isolates were Methicillin resistant *Staphylococcus aureus* (MRSA), which is much similar to other studies performed in India (Rouf and Nazir, 2019; Sharma *et al.*, 2017; Joshi *et al.*, 2013; Gopalakrishnan and Sureshkumar, 2010). There were no isolates of Vancomycin resistant *Staphylococcus aureus* (VRSA) or Vancomycin Intermediate *Staphylococcus aureus* (VISA). This may be due to cautious and controlled use of vancomycin in our hospital. Also in the present study 41.67% of *Enterococcus* isolates were found to be Vancomycin Resistant *Enterococcus* (VRE). However, in other studies *Enterococcus* showed 100% susceptibility to vancomycin (Rouf and Nazir, 2019; Sharma *et al.*, 2017; Deb *et al.*, 2014).

The preponderance of sterile body fluids infection and the AST pattern of each bacterial isolate varies in different settings. The current shift is an ever-increasing speed of antibiotic resistance in both gram negative and gram-positive isolates. It calls for a strict and organised surveillance and monitoring in

hospitals so the physicians can immediately acquire latest details on prevalence and antimicrobial susceptibility pattern which helps in shaping their clinical therapeutic judgement.

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### Conflicts of interest

Authors have no conflicts of interest to declare.

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