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Spectrum of Bacterial Isolates and their Antibiogram Isolated from Pus Sample

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A B S T R A C T

This study was conducted to know the spectrum of bacterial isolates from the pus samples and their sensitivity pattern so that it will help the clinicians to treat patients and help the hospital to have its antibiotic policy. A total 221 pus samples were collected and were processed in the laboratory using standard microbiological procedures. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method and results were interpreted. Out of 221 pus samples 200 (90.49 %) samples yielded a positive culture whereas 21(9.50%) samples yielded no growth. Among 200 samples, 112 (56%) were male patients and 88 (44%) were female patients. The most predominant Gram positive cocci isolated was *Staphylococcus aureus* 52(27.65%) and predominant Gram negative bacteria was *Pseudomonas* spp 40 (21.27%) apart from other isolates such as *Citrobacter* spp12 (6.38%), *Escherichia coli* 24(12.76%), *Klebsiella* spp 28(14.89%), *Proteus* spp 12(6.38%), Methicillin resistant coagulase negative staphylococcus16 (8.51%) and *Acinetobacter* spp 16 (8.51%) were also isolated. ESBL was found in 28 (21.21%) and MRSA 15(28.84%). Gram positive cocci shows 100% sensitivity to Vancomycin followed by Doxycycline. Whereas Gram negative bacilli were most susceptible to Imipenem followed by Amikacin and Gentamicin.

Introduction

Infectious diseases remain the most common cause of morbidity and mortality worldwide.

Pyogenic infections are characterized by local and systemic inflammation usually with pus formation. These may be endogenous or exogenous. A break in the skin can provide entry to the surface bacteria which thereby starts multiplying locally. The body's defence mechanism includes bringing immune cells into the area to fight against bacteria. Eventually, accumulation of these cells produces pus which is a thick whitish liquid (Koneman *et al*., 2005; Chopra *et al*., 1994). Extensive and sometime, unnecessary use of antimicrobial agents has resulted antibiotic resistance by development of antibiotic resistant genes in many organisms (Sengupta *et al*., 2001). Emergence of MRSA and ESBL producing

strains have been observed which confer resistance to commonly used drugs and poses a significant problem in deciding empiric therapy (Shenoy *et al*., 2010; Palit *et al*., 2010). Due to these emerging multi drug resistant strains there is need for continuous surveillance of such changing trend of antibiotics and updates knowledge to avoid the unguided empirical treatment. The present study was undertaken to analyze the pattern of pathogens and their antibiotic sensitivity isolated from pus.

Materials and Methods

The study was conducted from Jan 2015 to June 2015 in tertiary care hospital, Pune. A total 221 pus samples from different departments of tertiary care hospital, Pune were collected using aseptic technique. The specimen were inoculated on Blood agar and Mac-Conkey agar and incubated aerobically at 37˚C overnight.The isolates were identified by Gram staining, colony morphology and standard biochemical tests:- Catalase, Coagulase, Oxidase, Indole production, Methyl red test, Voges-Proskauer test, Citrate utilization (IMViC tests), H_2S production, Urease, Nitrate reduction test and Sugar fermentation tests (Collee *et al*., 2012).

Antimicrobial susceptibility testing

Antibiotic susceptibility tests were done on these isolates using Mueller-Hinton agar by standard Disc diffusion method according to CLSI guidelines. The following antibiotics were tested.

Amikacin (30mcg), Ampicillin (30mcg), Cefotaxime (30mcg), Co-trimoxazole (25mcg), Doxycycline (30mcg), Gentamicin (10mcg), Imipenem (10mcg), Piperacillin (100mcg), Ciprofloxacin (5mcg), Vancomycin (10mcg), Penicillin (10units), Erythromycin (15mcg), Cefoxitin for detection of MRSA and MRCONS (CLSI, 2012).

Detection of ESBL

This was performed by double disc diffusion method. Test organism were inoculated on Mueller hinton agar. The ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg / 10 µg) were placed at a distance of 20 mm apart on the agar. An increase of ≥5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer (Shiju *et al*., 2010).

Results and Discussion

Out of 221 pus samples 200 (90.49 %) samples yielded a positive culture whereas 21(9.50%) samples yielded no growth. Among 200 samples, 112 (56%) were male patients and 88 (44%) were female patients. The most predominant Gram positive cocci isolated was Staphylococcus aureus 52(27.65%) out of which 15(28.84%) was MRSA. Most predominant Gram negative bacteria was Pseudomonas spp 40 (21.27%) apart from other isolates such as *Citrobacter* spp 12(6.38%), *Escherichia coli* 24(12.76%), *Klebsiella* spp 28(14.89%), *Proteus* spp 12(6.38%), Methicillin resistant coagulase negative staphylococcus 16 (8.51%) and *Acinetobacter* spp 16 (8.51%) were also isolated.

ESBL was found in 28(21.21%). Maximum ESBL producers was observed in *E. coli* (37.05%) followed by *Klebsiella* spp.(28.57%), *Citrobacter* spp and *Proteus* spp (16.66%). While in *Acinetobacter* spp and Pseudomonas spp (12.05%) it was found to be minimum.

The sensitivity of Gram positive cocci shows that Vancomycin (100%) was the

most susceptible drug followed by Doxycycline(88.23%).Gram negative bacilli were most susceptible to Imipenem (100%) followed by Amikacin and Gentamicin (82.60%). Pseudomonas spp were also susceptible to Imipenem (100%) and Gentamicin (80%).

The emergence of resistant strains obtained from pus samples is highly threatening. Organisms associated with pyogenic infection were *Staphylococcus aureus*, *Pseudomonas* spp, *Escherichia coli*, *Klebsiella* spp. In our study, *Staphylococcus aureus* was most common Gram positive cocci while in Gram negative bacilli Pseudomonas was most predominant organism which is supported by Duggal *et al*., Raghav rao *et al*., and Sowmya *et al*., (2015, 2014, 2014) but contrary to the study of Soumya *et al*., (2014) were *Staphylococcus aureus* was most common Gram positive cocci and *E.coli* was most common Gram negative bacilli.

In our study Staphylococcus aureus (27.65%) was the most common gram positive cocci as shown in studies of Duggal *et al.*, (2015) and Raghav rao *et al.*, (2014). While MRSA (28.84 %) was shown similar to the study of Soumya *et al.*, (2014) *Pseudomonas* (29.73%) was the predominant gram negative bacilli which was similar to the Duggal *et al.*, and Raghav rao *et al.*, studies (2015, 2014). Majority of the cases in present study were males (56%) which is similar to the study conducted by Duggal *et al*., were male was (57.66%) (2015).

Table.1 ESBL producers among different isolates

Organisms	**Total no.of isolates**	**% of ESBL producers**
***Pseudomonas* spp (n=40)**	05	12.05
***Citrobacter* spp (n=12)**	02	16.66
***Esch.coli* (n=24)**	09	37.05
***Klebsiella* spp (n=28)**	08	28.57
***Proteus* spp (n=12)**	02	16.66
***Acinetobacter* spp (n=16)**	02	12.05
Total (n=132)	**28**	**21.21**

The majority of the ESBL producers were *E. coli* (37.05%) followed by *Klebsiella* spp(28.57%).

Table.2 Antibiotic Susceptibility pattern of Gram positive cocci

Organism	**Staphylococcus aureus and Coagulase Negative Staphylococci (n=68)**			
	Sensitive		**Resistant**	
Antibiotics	**Number**	**Percentage**	**Number**	**Percentage**
Co-trimoxazole	24	35.29	44	64.70
Cefoxitin	37	54.41	31	45.58
Penicillin	56	82.35	12	17.64
Doxycycline	60	88.23	08	11.76
Ciprofloxacin	39	57.35	29	42.64
Erythromycin	36	52.94	32	47.05
Vancomycin	68	100	00	00

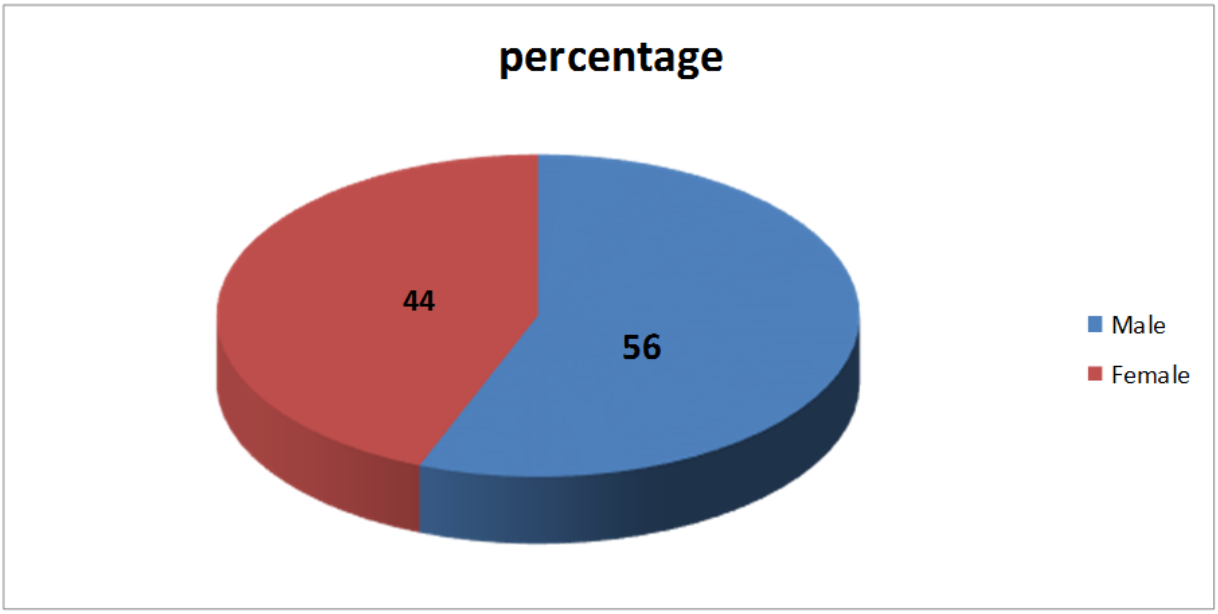
Table.3 Antibiotic Susceptibility pattern of Gram Negative bacilli other than *Pseudomonas* spp.

Organism	Enterobacteriaceae and *Acinetobacter* spp (n=92)			
	Sensitive		Resistant	
Antibiotics	Number	Percentage	Number	Percentage
Amikacin	76	82.60	16	17.39
Ampicillin	08	08.69	84	91.30
Cefotaxime	64	69.56	28	30.43
Co-trimoxazole	41	44.56	51	55.43
Gentamicin	76	82.60	16	17.39
Doxycycline	72	78.26	20	21.73
Ciprofloxacin	28	30.43	62	67.39
Imipenem	92	100	00	00

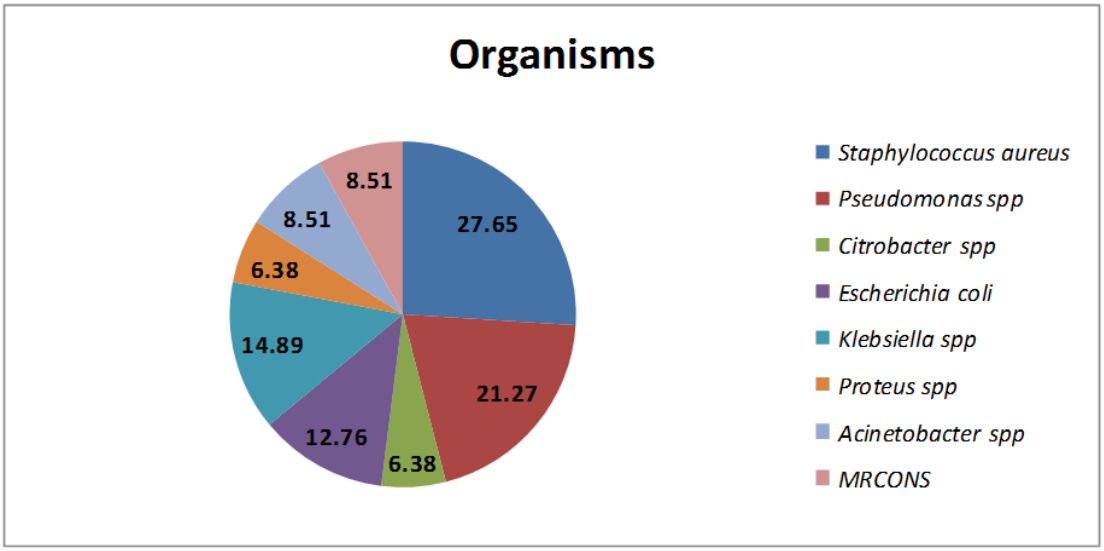
Table.4 Antibiotic Susceptibility pattern of *Pseudomonas* spp.

Organism	*Pseudomonas* spp (n=40)			
	Sensitive		Resistant	
Antibiotics	Number	Percentage	Number	Percentage
Amikacin	28	70	12	30
Gentamicin	32	80	08	20
Ciprofloxacin	28	70	12	30
Piperacillin	24	60	16	40
Ceftazidime	20	50	20	50
Imipenem	40	100	00	00

Graph.1 Sex-wise distribution of positive cultures obtained from pus samples



Graph.2 Pie chart showing various bacterial isolates obtained from pus samples



Most predominant Gram positive cocci was *Staphylococcus aureus* and Gram negative bacilli was *Pseudomonas* spp**.**

Staphylococcus aureus was susceptible to Vancomycin (100%) was similar to Raghav rao *et al.,* (2014) contrary to Duggal *et al.,* study (2015). Antibiotic sensitivity profile of gram negative bacteria showed sensitivity towards imipenem (100%), and Gentamicin (48.39%) as in studies of Raghav rao *et al.,* and Duggal *et al.,* (2015, 2014) but different in study conducted by sowmya *et al.,* were gram negative bacteria shows 100% sensitivity to Imipenem followed by Ciprofloxacin (73%) (Sowmya *et al*., 2014). ESBL production was seen in (21.21%). The ESBL production was found to be *E. coli* (37.05%) followed by *Klebsiella* spp. (28.57%), *Citrobacter* spp and *Proteus* spp (16.66%) which was similar to the result obtained Afroz *et al.,* (2014).

In conclusion, pyogenic infection has been major cause of morbidity. The changing trends of antimicrobial susceptibility in bacterial isolates from pus can serve as a useful tool for physicians to start empirical treatment of patients at the earliest according to the geographical areas and emerging multi-resistant bacteria**.**

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