Original article:

Evaluation of antimicrobial sensitivity and resistance pattern of Gram positive and Gram negative bacteria to monitor drug resistance among clinical isolates

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Abstract:

Introduction: Antibiotic therapy is usually given to protect from infection against various organisms, however, routine use of empirical treatment has resulted in widespread antibiotic resistance and development of antibiotic resistant genes.

Objective: To identify the bacterial pathogens isolated from various clinical specimens and to determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotics.

Materials & Methods: All the clinical specimens submitted to microbiology laboratory were processed as per standard microbiological procedures and antibiotic sensitivity tests were performed on the isolates as per guidelines of clinical and laboratory standards institute (CLSI).

Result: A total of 154 organisms were isolated from all clinical specimens, out of which 82 were Gram positive and 72 were Gram negative bacteria. Doxycycline (63.4%) and amikacin (84.4%) showed high degree of sensitivity among Gram positive bacteria. Amikacin (77.8%) showed high degree of sensitivity in Gram negative bacteria. Gram positive bacteria showed highest degree of resistance to penicillin (63.4%), whereas, Gram negative bacteria showed highest degree of resistance to cefazolin (73.6%).

Conclusion: High frequency of resistance against commonly used antibiotics such as penicillin and cefazolin as shown in the present study indicates a serious problem in the treatment of infections by gram positive and negative organisms. Therefore continuous surveillance is needed and treatment based on antibiogram report is essential.

Keywords:- Gram positive bacteria, Gram negative bacteria, Antibiotic sensitivity pattern, Antibiotic resistance pattern, Mueller Hinton Agar.

Introduction

Antibiotic therapy is usually given to protect from infection against various organisms and a wide generation of antibiotics are available for treatment. Prophylactic antibiotics play a significant role in the control of infections. However routine use of empirical treatment in both medical and veterinary medicine has resulted in widespread antibiotic resistance and development of antibiotic resistant genes.[1]

There is a continuous rise in the problem of antibiotic resistance throughout the world. Antimicrobial (AM) resistance is a serious clinical problem especially in intensive care units (ICUs), including critical care (CCU), neonatal and intensive cardiac care unit. Antimicrobial resistance in both Gram- negative and Gram-positive bacteria is commonly reported in hospital-acquired infections. Such drug resistance compromises the management of acute respiratory infections, sexually transmitted diseases and diseases spread by the fecal–oral route, such as typhoid fever, cholera, dysentery and other diarrheal diseases .[2-5]

In developed countries constant examination in this field has helped in recognizing antibiotic resistance pattern. Also, it has been reported that the spectrum and resistance of the pathogenic bacteria have constantly changed year after year because of extensive application of antimicrobial drugs. Widespread use of broad-spectrum antibiotics is the most important factor responsible for drug resistance. To overcome this problem and to improve the outcome of serious infections in our institution, monitoring of resistance patterns among clinical isolates in the hospital is needed. Also, it has been found that proper designing and follow up of Antibiotic control policy plays a major role in controlling drug resistance.[2,3,6-11]

Therefore, keeping the above mentioned things in mind the present study was done to identify bacterial pathogens isolated from various clinical specimens and determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotics.

Materials & methods

A hospital based prospective study was conducted from August to October 2015, and various clinical specimens, such as, urine, pus, sputum, blood, synovial fluid, bone, high vaginal swab and ear swab, submitted to the department of microbiology were included in the study.

All the collected specimens were cultured on blood agar and MacConkey agar and incubated aerobically at 37^{o}C for 24 – 48 hours, but in case of urine the culture was done on Cystiene lactose electrolyte deficient agar (CLED) and the plates were incubated for 24 hours at 37^{o}C aerobically. Growth on culture plates were identified by culture characteristics, gram's staining and standard biochemical test.[12]

The antimicrobial susceptibility test was performed on Mueller Hinton agar (Blood agar in case of *Streptococcus pyogenes*) by Kirby Bauer Disc diffusion method[13], and zone diameters in millimeters were recorded after incubation at 37^{o}C for 24 hours as per guidelines of clinical and laboratory standards institute (CLSI) using antibiotic discs (HiMedia Laboratories, India) such as, amikacin (30µg), gentamicin (10µg), clindamycin (2 µg), levofloxacin (5 µg), chloramphenicol (30 µg), cefoxitin (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), pristinamycin (15 µg), tobramycin (10 µg), erythromycin (15 µg), netilmicin (30 µg), penicillin (10 units), co-trimoxazole (1.25/23.75 µg), ampicillin (10 µg), high strength gentamicin (120 µg), high strength streptomycin (300 µg), vancomycin (30 µg), linezolid (30 µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone

(30µg), cefepime (30µg), cefaclor (30 µg), cefixime (5 µg),cefuroxime (30 µg), cefazolin (30 µg), imipenem (10µg), aztreonam (30 µg), doxycycline (30µg), fosfomycin (200 µg), norfloxacin (10µg), nitrofurantoin (300µg) and colistin (10 µg). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard quality control strains.[14]

Result

A total of 636 samples were included in the study, out of which 154 showed positive bacterial growth and 482 were negative for any bacterial growth.

Out of 154 isolated organisms, 82 were gram positive and 72 were gram negative bacteria [Figure 1]. As shown in Figure 2, maximum organisms were isolated from pus (58.4%) followed by urine (29.9%). Gram positive bacteria isolated were *Staphylococcus aureus* (40.2%), *Staphylococcus epidermidis* (1.3%), *Streptococcus pyogenes* (2.6%) and *Enterococcus* species (9.1%), whereas gram negative bacterial isolates were *Escherichia coli* (29.9%), *Pseudomonas aeruginosa* (5.2%), *Citrobacter* species (2.6%), *Acinetobacter* species (1.3%) and *Klebsiella* species (7.8%) [Table1, Figure 3].

Antibiotic sensitivity test of all the clinical isolates was performed as per CLSI and sensitivity pattern was noted. Doxycycline (63.4%) and amikacin (84.4%) showed high degree of sensitivity, whereas, penicillin (63.4%) showed high degree of resistance among gram positive bacteria. Amikacin (77.8%) showed high degree of sensitivity, whereas, cefazolin (73.6%) showed high degree of resistance in gram negative bacteria [Table 2 and 3].

Table 1: Distribution pattern of isolated organisms according to specimens

ORGANISMS ISOLATED	SAMPLES TESTED								TOTAL
	Urine	Pus	Sputum	Blood	Synovial Fluid	Bone	High Vaginal Swab	Ear Swab	
Staphylococcus aureus	4	48	2	-	2	-	4	2	62
Staphylococcus epidermidis	-	2	-	-	-	-	-	-	2
Streptococcus pyogenes	-	2	2	-	-	-	-	-	4
Enterococcus species	10	4	-	-	-	-	-	-	14

Escherichia coli	28	16	-	-	-	-	2	-	46
Pseudomonas aeruginosa	-	4	-	2	-	2	-	-	8
Klebsiella species	4	8	-	-	-	-	-	-	12
Citrobacter species	-	4	-	-	-	-	-	-	4
Acinetobacter species	-	2	-	-	-	-	-	-	2
TOTAL	46	90	4	2	2	2	6	2	**154**

Table 2: Antibiotic sensitivity pattern of gram positive isolates

ANTIBIOTICS TESTED	**ORGANISMS (n=82)**							
	Staphylococcus aureus (n=62)		*Staphylococcus epidermidis* (n=2)		*Streptococcus pyogenes* (n=4)		*Enterococcus* species (n=14)	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Amikacin	52	10	2	0	NT	NT	NT	NT
Clindamycin	40	22	2	0	4	0	NT	NT
Doxycycline	42	20	0	2	4	0	6	8
Levofloxacin	34	28	2	0	4	0	NT	NT
Chloramphenicol	36	26	2	0	NT	NT	8	6
Cefoxitin	38	24	2	0	NT	NT	NT	NT
Ofloxacin	32	30	2	0	3	1	NT	NT

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Ciprofloxacin	32	30	2	0	NT	NT	NT	NT
Gentamicin	32	30	0	2	NT	NT	NT	NT
Pristinamycin	38	24	2	0	NT	NT	4	10
Tobramycin	34	28	2	0	NT	NT	NT	NT
Netilmicin	34	28	2	0	NT	NT	NT	NT
Erythromycin	22	40	2	0	4	0	8	6
Penicillin	18	44	0	2	2	2	10	4
Co-trimoxazole	24	38	0	2	NT	NT	NT	NT
Ampicillin	28	34	0	2	0	4	12	14
High strength Streptomycin	NT	NT	NT	NT	NT	NT	10	4
High strength Gentamicin	NT	NT	NT	NT	NT	NT	11	3
Vancomycin	NT	NT	NT	NT	4	0	12	2
Linezolid	62	0	2	0	NT	NT	14	0
S= sensitive; R= resistant; n=number of organisms; NT= Not tested.								

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Table 3: Antibiotic sensitivity pattern of gram negative isolates

ANTIBIOTICS TESTED	ORGANISMS (n=72)									
	Escherichia coli (n=46)		*Pseudomonas aeruginosa* (n=8)		*Klebsiella* species (n=12)		*Citrobacter* species (n=4)		*Acinetobacter* species (n=2)	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Amikacin	32	14	8	0	10	2	4	0	2	0
Doxycycline	14	32	NT	NT	2	10	2	2	2	0
Levofloxacin	16	30	8	0	4	8	3	1	2	0
Cefoxitin	26	20	NT	NT	1	11	1	3	NT	NT
Ofloxacin$^{\alpha}$	8	38	4	4	2	10	3	1	NT	NT
Ciprofloxacin	10	36	2	6	4	8	2	2	0	2
Gentamicin	16	30	6	2	6	6	2	2	2	0
Ampicillin	18	28	NT	NT	NT	NT	0	4	NT	NT
Cefotaxime	10	34	NT	NT	2	10	3	1	0	2
Ceftazidime	8	38	6	2	3	9	2	2	2	0
Ceftriaxone	10	36	NT	NT	2	10	3	1	1	1
Cefepime	16	30	8	0	4	8	2	2	2	0
Cefaclor	12	34	NT	NT	1	11	0	4	NT	NT
Cefixime	14	32	NT	NT	3	9	3	1	NT	NT
Cefuroxime	6	40	NT	NT	2	10	2	2	NT	NT
Cefazolin	8	38	NT	NT	1	11	0	4	NT	NT

Piperacillin	20	26	3	5	4	8	2	2	0	2
Piperacillin-Tazobactam	24	22	8	0	6	6	3	1	1	1
Fosfomycin[β]	28	18	NT	NT	NT	NT	NT	NT	NT	NT
Nitrofurantoin[γ]	30	16	NT	NT	8	4	4	0	NT	NT
Norfloxacin[α]	24	22	4	4	6	6	2	2	NT	NT
Aztreonam	22	24	6	2	2	10	1	3	NT	NT
Imipenem	44	2	6	2	11	1	4	0	1	1
Colistin[£]	NT	NT	4	4	NT	NT	NT	NT	NT	NT

S= sensitive; R= resistant; n=number of organisms. NT = Not tested.

[α]tested for urinary isolates only; [β]tested for urinary isolates of *Escherichia coli* only; [γ]tested for urinary isolates of *Enterbacteriaceae* only; [£]tested for *Pseudomonas aeruginosa* only.

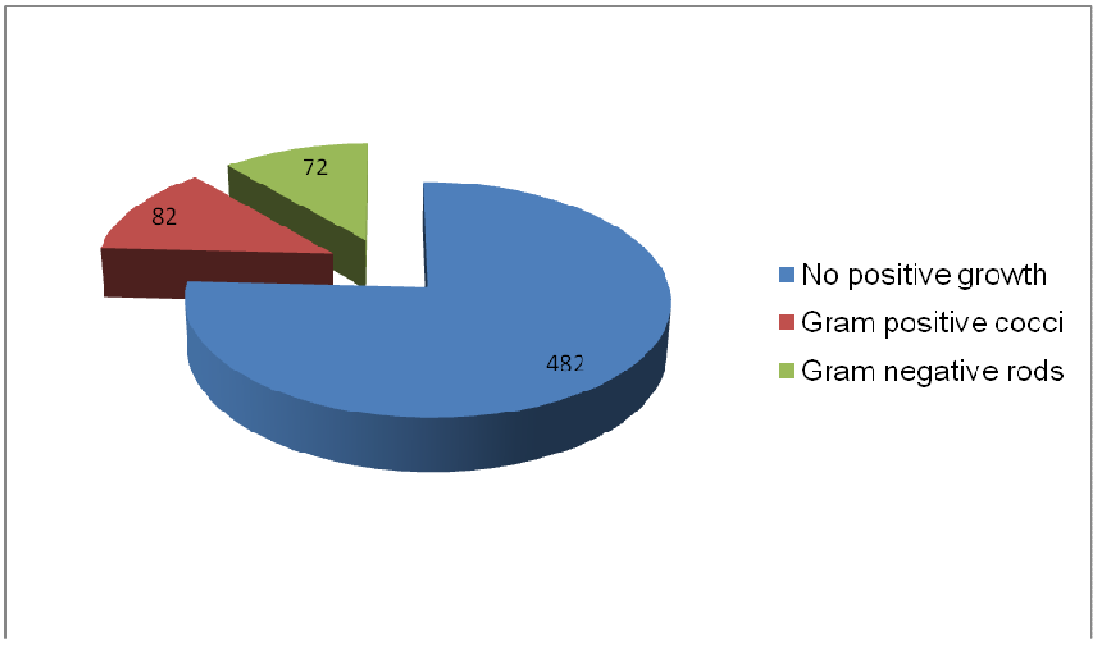

Figure 1: Distribution of positive and negative growth in all clinical specimens.

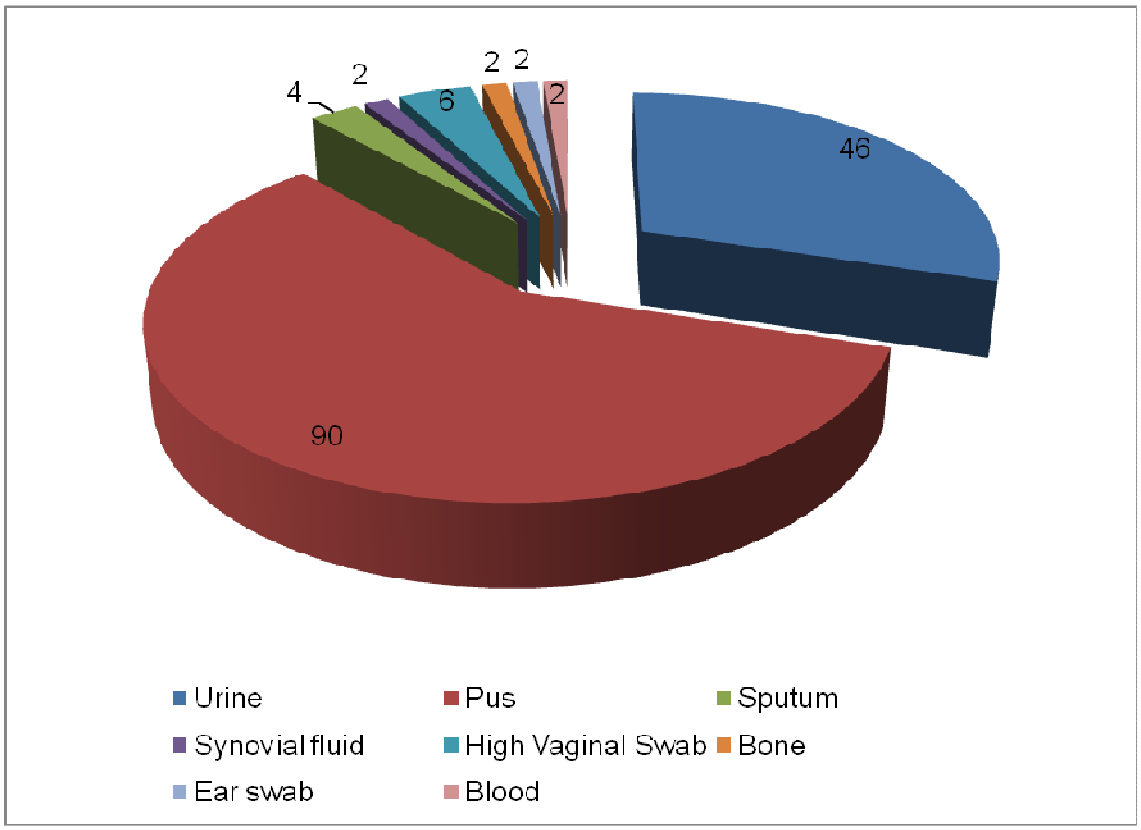

Figure 2: Distribution of various specimens which were showing positive growth.

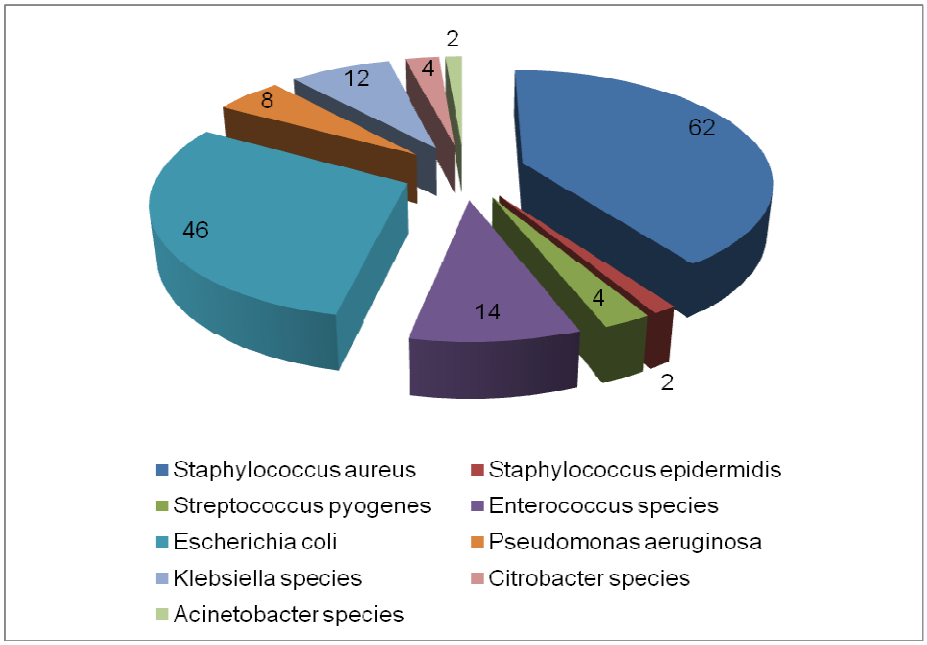

Figure 3: Distribution of various isolated organisms from clinical samples.

Discussion

The discovery of antibiotics revolutionized the management of infectious diseases. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to these life saving drugs. Resistance due to adulteration of the antibiotics has also been reported. The microbial pathogens, as well as their antibiotic sensitivity patterns may change from time to time and place to place. Hospital antibiograms are commonly used to help guide empirical antimicrobial treatment and are an important tool for detecting and monitoring trends in antimicrobial resistance.[15,16] Keeping this in mind the present study was done to evaluate the sensitivity and resistance pattern of various clinical isolates.

A total of 636 samples were submitted in the microbiology laboratory, out of which 154 showed positive bacterial growths. In the present study maximum clinical isolates were from pus (58.4%) followed by urine (29.9%). The prevalence of gram positive cocci was higher (53.2%) than the gram negative rods (46.7%). These findings are similar to those of other worker who also reported higher growth of gram positive bacteria (51%) as compared to gram negative bacteria (49%) from clinical samples.[17-20]

Amongst the gram negative isolates in our study most of them were found to be sensitive to amikacin (77.8%), piperacillin-tazobactam (58.3%) and imipenem (91.7%) and maximum resistance was shown to cefazolin (73.6%). This finding is similar to another study which also showed maximum sensitivity of gram negative bacteria to amikacin (87.8%), piperacillin-tazobactam (79.7%) and imipenem (78.3%).[20]

In the present study, all the *Pseudomonas aeruginosa* isolates were found to be 100% sensitive to amikacin, piperacillin-tazobactam and cefepime, followed by sensitivity to imipenem (75%), ceftazidime (75%) and colistin (50%). This is similar to another study which showed highest sensitivity of *Pseudomonas aeruginosa* isolates to amikacin (68.01%) followed by ceftazidime (57.08%), however, in contrast to our study, they reported 100% sensitivity to imipenem.[19]

In our study the most prevalent gram positive bacteria was *Staphylococcus aureus* (40.2 %) followed by *Enterococcus* species (9.1%), which is comparable to another study done previously.[21]

In the present study it was found that most of the isolates of *Staphylococci* were highly sensitive to amikacin (84.4%). Amongst other tested drugs doxycycline (63.4%) showed high sensitivity among gram positive bacteria, whereas, most of them were found to be resistant to penicillin (63.4%). This finding is similar to another study which also reported high susceptibility of *Staphylococcus* to amikacin.[22]

In our study it was seen that the isolates of *Enterococcus* species were highly susceptible to penicillin (71.4%), vancomycin (85.7%) and linezolid (100%). Also, these isolates showed high susceptibility to both high strength gentamicin (78.6%) and high strength streptomycin (71.4%), therefore, combination treatment with penicillin / vancomycin and aminoglycosides can be given to treat infection effectively.

Conclusion

High frequency of resistance against commonly used antibiotics such as penicillin and cefazolin as reported in the present study indicates a serious problem in the management and treatment of infections caused by gram positive and negative organisms. To overcome this problem of drug resistance, continuous surveillance is needed and treatment based on antibiogram report is essential.

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