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Antibiotic Susceptibility of Various Clinical Samples: Tool for Prevention of Infectious Diseases

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ABSTRACT:

Many different studies conducted at many places have shown predictable bacterial strain profiles and their antibiotic resistance patterns. These studies has also reported the capability of microbes to accept the changes in the environment for their survival, which leads to the rise in all kind of infections including hospital acquired infections. Therefore, regular observation and check in the bacterial profiles and their antimicrobial resistance are needed.

In this study antibiotic susceptibility of various clinical isolates was tested by Kirby Beaur method. The results of the study showed a high degree of resistance to various antimicrobial agents, which is a matter of concern.

KEY WORDS: antibiotic susceptibility, surveillance, emergence, antimicrobial, resistance

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INTRODUCTION:

Persistence of pathologic processes of an organism that are resistant to first line antibiotics is state of worrisome. [1] The emergence is associated with high mortality and morbidity, which not only impacts on patients but raises the burden of health care systems. [2] Although the main causes of resistance are well understood but precise usage of drugs has yet to be fully understood. [3] Thus an approach to surveillance can be made by antimicrobial susceptibility testing. [4, 5]

Since the primary role of determining the susceptibility of an organism is to lead or direct the care and treatment of the patient, the antibiotics selection for surveillance needs to take this into consideration. [6] The motive of surveillance is to put collected information for action to arrest or intercept the antimicrobial resistance spreading everywhere. Thus the approach of surveillance is determined by nature and timeliness with which the information is required. In antimicrobial surveillance, it is important to make real distinctions between the real emergences due to pathogens or it is high because of opting new methods or sample collection techniques.

METHODS AND MATERIALS:

The collection and processing of specimen for this purpose should be undertaken in consistent way and the appropriate quality standard. Wherever possible, the procedure of obtaining specimen should be readily understood and acceptable to patients. [7]

The present work deals with the antibiotic sensitivity and surveillance study of pathogenic organisms isolated from clinical samples of Nawanshahr region of Punjab by our research team [8]

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antibiotic susceptibility testing was done by Kirby beaur method. [9] The isolated colony of bacteria was transferred to a test tube containing 1.5 ml sterile saline. The density of the suspension was visually equivalent to the barium sulphate standard, 0.5 Mc farland units. Before use, the standard should be shaken vigorously. A cotton swab was dispensed into the suspension and removed by rotation of the swab against the side of the tube. [10] The medium was inoculated by even streaking of the swab over the entire surface of the plate in three directions. After the inoculum has dried, discs were applied with the forceps, a dispenser and pressed gently to ensure even contact with the medium. [11]

The antibiotics panel that was tested for each organism was according to CLSI guidelines. The organism was defined as sensitive, intermediately sensitive and resistant by breakpoints defined by CLSI. [12]

The antibiotics that were used for GPC's were Penicillin, Oxacillin, Amoxicillin-Clavilinic Acid, Vancomycin, Teicolanin, Clindamycin, Cotrimoxazole, Erythromycin, Linezolid, Cefoxitin And Tetracycline. For GNB's Gentamycin, Amikacin, Netilmycin, Tobramycin, Ceftriaxone, Caftazidime, Gatifloxacin, Ciprofloxacin, Ampicillin, Imipenem, Cotrimoxazole, Ticarcillin, Polimixin –B, Piperacillin-Tazobactem, Cefaparazone-Sulbactem were used. [12]

RESULTS:

During a two year study in Nawanshahr (2012-2014), total number of 111(12.2%) pathogenic strains was isolated from 908 clinical samples including urine, blood, pus and stool. (Figure 1.) Among 111 isolates, the isolates from adult male patients were 36%, whereas, the positivity rate of adult females was 46%. However, positivity rate of children both male and female was very less in comparison to adults being 3.2% and 4.8% respectively. (Table1.) The pathogens were identified by various morphological and biochemical properties. Among 111 strains of various microorganisms 51 (45.94%) were gram positive cocci and 60 (54.05%) were identified to be as gram negative bacilli. the frequencies of both GNB and GPC varied depending on type of clinical sample. The most prevalent pathogen in all samples found to be was Enterococcus *spp.* that contributed 30.6% to all infections. The second prominent pathogen was found to be Escherichia *coli* (23.4%). However, other gram negative bacilli like Citrobacter *spp.*, Enterobacter *spp.* and Proteus *spp.* contributed least to all infection (2.7%). overall, 15.3% infections were due to Staphylococcus *aureus*, whereas, in rest of gram negatives, only 5.4% were due to Klebsiella *spp.*, 6.3% were due to Pseudomonas *spp.* and 7.2% were due to Acinatobacter *spp.*(Table 2.)

The antibiotic susceptibity testing by Kirby Beaur method (Figure 2.) revealed that out of 51 GPC's isolated, 50 (98.03%) was resistant to antibiotic Penicillin. But the results of Penicillin combinations (Augmentin) were different. Only 21.56% GPC found to be resistant to Augmentin. As studies on antimicrobial resistance has shown rising resistance of microbes to macrolides since long, here also it left the same impact with 49% resistant cases. Higher antibiotics like Vancomycin, Linezolid, and Teicoplanin were 100% sensitive. Antibiotics like Clindamycin also showed low resistance rate i.e., 17.6%. MRSA identification by both antibiotics Cefoxitin and Oxacillin was 17.6% and 15.6% respectively. Resistance to sulphonamides like Cotrimoxazole was 60.7%.whereas,out of 60 GNB (lactose fermenters) isolated resistance rate to sulphonamide antibiotic (Cotrimoxazole) was very high(90%). for Cotrimoxazole, Acinatobacter was 100% resistant and Pseudomonas was 71.4%.(table 3.)

Penicillins (Penicillin and Ampicillin) that were used for GNB and GPC showed different degree of resistance. In case of GPC where Penicillin was used resistance rate was 90% but in case of GNB where Ampicillin was used the resistant rate was quite low comparatively, liming to 50%.

For gram negative bacilli, out of 60 GNB 45% were resistant to gentamycin, 31.66% were to Amikacin, and 4.3% to Netilmycin. Out of three Cephalosporins used there, the most effective Cephalosporin found to be was Ceftriaxone to which only 26 GNB showed resistance. And 92% of GNB showed resistance to ceftazidime. Results of cefotaxime resistance were also surprising indicating 38% of pathogen resisted to the antibiotic. For fluoroquinolones, like Gatifloxacin, resistance rate was quite low, i.e., 18%. Same was for ciprofloxacin(16%).

resistance to Carbepenem (imipenem) was also surprising being 36%, resistance to piperacillin tazobactem was 82% proving that Penicillin combinations are more effective in GPC and Penicillins alone better work in GNBs. Resistance to other combinations like Cefaparazone sulbactem was 58%.(Table 4.)
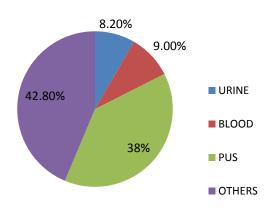

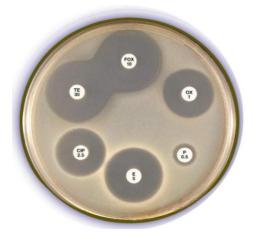**Figure 1. Relative percentage of positive samples.**

Figure 2. Kirby Beauer method of Antibiotic susceptibility testing.

Table 1. Age and sex wise distribution of culture positive cases.

SEX	VARIOUS CULTURE POSITIVE CASES (n=125)
MALE	45 (36%)
FEMALE	70 (56%)
MALE CHLIDREN	4 (3.2%)
FEMALE CHILDREN	6 (4.8%)
TOTAL (n=125)	102 (100%)

Table 2. Aerobic bacterial strain isolated in each sample.

Type of isolate	Urine# (762)	Blood #(33)	Stool #(6)	Pus#(100)	Other body fluids #(7)	Grand tot al
Staphylo coccus aureus	7	2	0	7	1	17
Enteroco ccus spp.	18	0	0	14	2	34
Klebsiella spp.	4	0	0	2	0	6
Pseudom onas spp.	5	1	0	1	0	7
Acinatob acter spp.	5	0	0	3	0	8
Proteus spp.	0	0	0	3	0	3
Citrobact er spp.	0	0	0	3	0	3
E coli	22	0	0	4	0	26
Enteroba cter	2	0	0	1	0	3
Salmonel la typhimyr uium	0	0	4	0	0	4
Total each	63	3	4	38	3	

 # The values in parentheses reflect total number of clinical samples studied.

Almost GNB's were resistant to antibiotics like piperacillin tazobactem and Cefaparazone Sulbactem. To Piperacillin Tazobactem 100% of GNB showed resistance except Klebsiella spp. where the resistance to Piperacillin Tazobactem 83.3%. Only one Pseudomonas spp. was sensitive to Piperacillin Tazobactem.

In case of Cefaparazone Sulbactem again all GNB except Klebsiella spp. (66.6%) showed 100% resistance. Only one spp. of Acinatobacter showed sensitivity towards Cefaparazone- Sulbactem. Both of non-lactose fermenters (Pseudomonas and Acinatobacter) were 100% sensitive to Tobramycin, Colistin, Polymixin B and Aztreonam. In case of imipenem, pseudomonas was 100% sensitive, but 37.5% of Acinatobacter *spp.* were resistant to imipenem. Pseudomonas was resistant to Ampicillin. Both Pseudomonas *spp.* and Acinatobacter *spp.* showed resistance 42.8% and 50% respectively. Ceftazidime was 100% resistant in both. The resistance

rate towards Cefotaxime was 42.8% and 37.5% in Pseudomonas and Acinatobacter respectively. In case of Ceftriaxone, it was 28.5% and 37.5% for Pseudomonas and Acinatobacter. For gentamicin it was 28.57% and 87.5% in Pseudomonas and Acinatobacter *spp.* The study reflected that Pseudomonas showed same degree of resistance to Ciprofloxacin, Gatifloxacin and Amikacin (14.2%). Whereas for Acinatobacter *spp.* It was 25%, 25% and 37.5% respectively.

Table 3. Gram Positive cocci and their antibiotic resistance profile.

GRAM POSITIVE COCCI			
		Staph. Aureus (17)	Enterococcus(34)
PENICILLINS	PENICILLIN	16	34
	OXACILLIN	8	-
	AUGMENTIN	3	8
GLYCOPEPTIDE ANTIBIOTIC	VANCOMYCIN	0	0
	TEICOPLANIN	0	0
LINCOSAMIDES	CLINDAMYCIN	4	5
SULPHONAMIDES	COTRIMOXAZOLE	15	16
MACROLIDES	ERYTHROMYCIN	10	15
OXAZOLIDINONE	LINEZOLID	0	0
CEPHALOSPORINS	CEFOXITIN	9	-
TETRACYCLINES	TETRACYCLINE	-	6

According to the present study, GNB's are more responsible for any kind of infections in comparison to GPC's. But if observed individually, Enterococcus *spp.* was quite prominent in all clinical samples, even more than E.*coli* (23.4%). A prominent part of GPC is taken as S. *aureus* (15%). Non lactose fermenters like Pseudomonas and Acinatobacter showed higher rate of infection (6.3% and 7.2 % respectively). Proportion of nil fermenters is slightly higher than fermenters. Their susceptibility patterns were also striking. The influence on antibiotics on various pathogens has been noted so many times. [13]

DISCUSSION:

Ideally antimicrobial surveillance should include collection of clinical and epidemiological data. There is evidence that wiser use of antimicrobials may diminish the rate of emergence of resistance. [14] The surveillance studies have been conducted at so many places at large scales, but no significant studies have been made in Punjab. This study is oriented on various different antibiotic resistance patterns in Nawanshahr region of Punjab. The results of isolates show that there is not a big difference between the infection rates of both GPC and GNB. The little difference that has come out may be due to type of clinical samples. The present study reveals that Enterococcus *spp.* is the most commonly infecting pathogen in all types of clinical samples. The second most commonly occurring pathogen in infection was found to be E.*coli* that coincides with study by Rao *et al*. [14] There have been studies that show second dominance of GNB's in many kinds of clinical samples. [15, 16] Such GNB dominance in the aerobic growth in pus culture has been highly seconded by this study. Though antibiotic class Penicillins showed variable results between Penicillin and Ampicillin, their combinations are not up to satisfactory mark. According to this data, Penicillins should be kept aside in treating GPC infections in particular region. Though combination may work, but resistance genes may develop.

The resistance range of macrolides like erythromycin shows raising tolerance of GPC everywhere and has been observed in this study too. [9] Judicial and timely usage of erythromycin may be helpful in diminishing the resistance.

3rd generation Cephalosporins usage and their resistance has been a leading cause of extended spectrum beta lactamases (ESBL) production. The resistance has been

Table 4. Gram negative bacilli and their antibiotic resistance profile.

		KLEBSIELLA spp.(6)	E.coli(26)	CITROBACTER spp.(3)	ENTEROBACTER spp.(3)	PROTEUS spp.(3)	PSEUDOMONAS spp.(7)	ACINATOBACTER spp.(8)	Total 60
BACTERIAL STRAINS									
AMINOGLYCOSIDES	GENTAMYCIN	1	11	3	1	2	2	7	27
	AMIKACIN	2	12	1	0	0	1	3	19
	NETILMYCIN	0	2	0	0	1	0	2	5
	TOBRAMYCIN	-	-	-	-	-	0	0	
CEPHALOSPORINS	CEFTRIAXONE	1	4	1	1	1	2	3	13
	CEFOTAXIME	2	7	1	0	3	3	3	19
	CEFTAZIDIME	5	22	2	2	0	7	8	46
FLUOROQUINOLONES	GATIFLOXACIN	2	5	0	0	0	0	2	9
	CIPROFLOXACIN	0	4	0	1	0	1	2	8
PENICILLINS	AMPICILLIN	3	10	2	3		3	4	25
CARBEPENEMS	IMIPENEM	1	12	1	0	1	0	3	18
SULPHONAMIDES	COTRIMOXAZOLE	4	19	3	3	3	5	8	45
POLIMIXINS	COLISTIN	0	0	0	0	0	0	0	
ANTI PSEUDOMONAL PENICILLINS	TICARCILLIN	-	-	-	-	-	0	0	
	POLIMIXIN B	-	-	-	-	-	0	0	
MONOBACTEM	AZTREONAM	-	-	-	-	-	0	0	
OTHERS	PIPERACILLIN TAZOBACTEM	4	15	3	3	3	6	7	41
	CEFAPARAZONE SULBACTEM	5	0	3	3	3	7	8	29

 # The values in parentheses reflect total number of each bacterial strain studied.

noted worldwide but shockingly, [17] Ceftazidime showed resistance at very large scale i.e., 92%. The fluoroquinolones result was likely to be as expected.

CONCLUSION:

Surveillance is an aid that can smoothen the way to prevention of infection and can make better use of its instant and long-term upshots by furnishing the required particular, facts and other information for measures. Hence by creating surveillance systems that integrate clinical and laboratory data, not only can the required data be captured but the strengths of both data sets can be combined to combat with unnecessary existence antimicrobial resistance and can help public by reducing morbidity and mortality furthermore with good and healthy life. Even though the specific antibiograms are selected but there is a need of change in the antibiogram that are showing raised resistance. This can be achieved with the easy tools like surveillance of antibiotic resistance.

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