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Research Article

ANTIBIOGRAM TYPING OF GRAM NEGATIVE ISOLATES IN DIFFERENT CLINICAL SAMPLES OF A TERTIARY HOSPITAL

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ABSTRACT

Background: The knowledge of resistivity pattern of different clinical isolates of hospital has been the global necessity for control of emergence of resistance to antimicrobial agents. The varying pattern of use of therapeutic agents and lack of good stewardship has contributed to the increase in these multidrug resistant organisms. This study thus aims to provide the evidence of the various resistivity patterns of Gram negative isolates.Method: This study reports the antibiotic resistivity pattern of gram negative isolates from all clinical samples received in one of the tertiary hospital in capital of Nepal. Total of 1110 clinical samples both from OPD and hospital wards were received during six months study period which included Urine (555), Blood (445), Pus (75), other samples (35) including catheter swabs, sputum. The gram negative isolates were then subjected to Antibiotic susceptibility testing using Kirby Bauer Disc Diffusion technique with 11 different antibiotic discs.Result and Conclusion: Among sample tested (181, 16.3%) were found to be positive with 159 gram negative isolates from total positive sample. *E. coli* (113/181) was the major followed by *Salmonella* Typhi (17/181), *Klebsiella* spp. (14/181), *S.* Paratyphi (7/181), *Acinetobacter* spp. (5/181) and *Proteus* spp. (3/181). 83 of the isolates were found to be MDR with 60.2% (68/113) *E. coli*; 100 %(14/14) *Klebsiella* spp.; 29.4% (5/17) *S.* Typhi; 80.0% (4/5) *Acinetobacter* spp. and 33.3% (1/3) of *Proteus* spp. From antibiogram for *Acinetobacter* spp. Sulphonamides (Co-trimoxazole) and Cephalosporins (Cefotaxime and Ceftriaxone) was found to be most effective and Nitrofurantion was least effective, for *E. coli* Ciprofloxacin was most effective drug and Nitrofurantoin least effective, for *Klebsiella* spp. Co-trimoxazole, Ciprofloxacin and Gentamycin were found to be effective similarly for *S.* Typhi Chloramphenicol and Ciprofloxacin was found to be most effective drug.

Keywords: Antibiogram, Antibiotic Susceptibility test, Gram Negative, Multidrug resistant

INTRODUCTION

The threat to health care associated infection has been a matter of concern due to development of multidrug resistant (MDR) strains among common isolates in hospital. Applying antibiotics caused breakthrough in treatment stepping out in ill's group infections treated in hospital however enlarging resistance, the formation of new mechanisms of resistance, and/or the spreading of gene of resistance has become the shortcomings of antibiotic therapy.[1] The worldwide escalation in both community- and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and new treatment alternatives.[2-5] Antimicrobial-resistant pathogens, including methicillin resistant *Staphylococcus aureus* (MRSA) (community associated [CA-MRSA] and healthcare-associated [HA-MRSA]), vancomycin-resistant *Enterococcus* species (VRE), penicillin-resistant *Streptococcus pneumoniae*, extended-spectrum b-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* species,[(2-3)] and fluoroquinolone-resistant and carbapenem resistant members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* are increasing in prevalence globally. [2.6-7]

Resistant emerges from over utilization of antibiotics trying to sterilize the environment and also the inappropriate use of the antibiotics for treatment. Free availability and self medication of antibiotics, lack of access to health facilities, in adequate public awareness, uncontrolled antibiotics use in agriculture, lack of adequate antimicrobial resistance surveillance and lack of updated national antibiotic polices and guidelines are added worries. Antibiotics are commonly used in animals for prophylaxis or as performance enhances and such practices are likely to increase the development of resistance. [1]

Asia is regarded as one of the epicentres of antimicrobial drug resistance, there is an alarming number of antibiotic-resistant species, including MDR *A. baumannii*, extended-spectrum β-lactamase (ESBLs)-producing *K. pneumoniae* (particularly mediated by CTX-M-9, CTX-M-14 and CTX-M-15), New Delhi metallo-β-lactamase 1 (NDM-1) producing Enterobacteriaceae, MDR *S. enterica* serotypes Choleraesuis and Typhi, carbapenem-resistant *A. baumannii* (OXA-58 and OXA-23 carbapenemases.)[9] Continuous surveillance of resistance data from clinical isolates as well as implementation of strict infection control policies in healthcare settings are required to mitigate the progression. Marked variations in the resistance profiles of bacterial pathogens as well as the quality of public hygiene have had a considerable impact on the effectiveness of antimicrobial agents in Asian countries. [9-11] Nepal, of South Asia is also not too far in occurrence of increasing trend of resistant "superbug". Many cases of MDR gram negatives have been reported in Nepal.

METHODS AND METHODOLOGY

Study Population

All clinical samples received in microbiology laboratory were considered from all the units of the hospital

Sample Collection

Clinical samples which include mid stream urine, blood, sputum samples, catheter swabs were collected from prescribed patients. The samples were collected and labeled in medical laboratory unit of the hospital. These samples were analyzed within 30 minutes to 1 hour of collection.

Culturing, Characterisation and Identification of Bacteria[12-14]

Samples were processed according to the sample nature type. All the samples were cultured in MacConkey Agar, Blood agar plates and incubated at 37° C for 24 hours. The sample where heavy pure growth of pathogen was obtained was included in this study. Isolates were subcultured and colonies were screened for gram negative isolates. Identification of isolates were done by gram staining, catalase, oxidase, biochemical test Triple sugar Iron agar test, Citrate Utilization test, Indole test, Methyl red test, Voges Progeskaur test, Urease test. Result interpretation was done based on the identifying characters as described by Cheesbrough.

Antibiotic susceptibility Test [15]

Antibiotic susceptibility test of all isolates was performed by Kirby disc diffusion method. In this technique a disc impregnated with antimicrobial agents are laid over the carpet culture of test

organism, antimicrobial agent diffuses radially from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc. Bacterial strains sensitive to the antimicrobial were inhibited at a distance from the disc where as resistant strain grew up to the edge of the disc. Antibiotic disc Ampicillin (10 mcg/disc), Co-trimoxazole (25mcg/disc), Gentamycin (10 mcg /disc), Ciprofloxacin (5 mcg /disc), Cefotaxime (30 mcg/disc), Ceftriaxone (30mcg/ disc), Cefixime (5 mcg/ disc), Amoxicillin (10mcg/disc), Chloramphenicol (30 mcg/ disc), Amikacin (30 mcg/ disc), Nitrofurantoin (300 mcg/ disc) were used.

Interpretation of the results was done using the zone of inhibition sizes provided the antibiotic disc manufacturer (Hi-Media).

RESULT

Table 1 show the significant growth of the total of 1110 clinical samples (555 urine, 445 blood, 75 pus and 35 other samples) analyzed in the study period. 23.2% (129) Urine sample showed significant growth, 5.4% (24) Blood showed growth, and 33.3% (25) Pus showed growth and only 8.6% (3) of other samples showed significant growth. Thus 16.3% (181) clinical samples gave the positive result. Of the total 159 isolates identified as gram negative; which were further analyzed for antibiotic resistivity testing.

Table 1:Significant growth in total clinical samples and the organism pattern

S. No.	Sample	Total	significant growth	gram negative
1	Urine	555	129 (23.2 %)	125/129 (8.7%)
2	Blood	445	24 (5.4 %)	24/24 (100.0%)
3	Pus	75	25 (33.3 %)	7/25 (28.0%)
4	Others	35	3 (8.6 %)	3/3(100.0%)
Total		1110	181 (16.3 %)	159/181(88.4%)

Table 2 shows the different types of gram negative isolates obtained from different sample that includes most common isolates was *E. coli*, *S*. Typhi. , *Klebsiella* spp., *S*. Paratyphi, *Acinetobacter* spp., and *Proteus* spp. Among these the most predominant isolate from urine was *E. coli*. In blood majority were of *S*. Typhi.

Table 2:Distribution of bacterial isolates in different clinical samples

S. No.	Organism	Sample				Total
		Urine	Blood	Pus	Others	
1	*Acinetobacter* spp.	3	0	2	0	5(3.1%)
2	*E. coli*	108	0	2	3	113 (71.1%)
3	*Klebsiella* spp.	12	0	2	0	14 (8.8%)
4	*Proteus* spp.	2	0	1	0	3 (1.9%)
5	*S*. Paratyphi	0	7	0	0	7 (4.4%)
6	*S*. Typhi	0	17	0	0	17 (10.7%)
Total		125 (78.6%)	24 (15.1%)	7 (4.4%)	3 (1.9%)	159 (100.0%)

Table 3 shows the resistivity pattern of the gram negative isolates of total 159 gram negative isolates tested for antibiotic susceptibility test. Each organism showed the varied resistivity pattern of the gram negative isolates. The isolates were classified into seven different antibiotye groups denoted by roman numerals (shown in table 3). For all the isolated organisms type V antibiotype i.e. resistant to Nitrofurantoin is most common. Besides for *Acinetobacter* spp. type IV Type of antibiotype is found to be most occurring resistant type while in case of *E. coli* VI and IV Types of antibiotype group is found to be highly resistive. Type I i.e resistance to Aminopenicillins (amoxicillin) is seen high among the *Klebsiella* spp. and in case of *S*. Typhi. While no resistances were seen among the *S*. Paratyphi organisms isolated during the study period.

Table 3:Antibiotype classification of gram negative isolates from clinical sample

Antibiotype Division	Resistant Antibiotic Group	Resistant Antibiotic	[a]Antibiotic Resistivity Pattern (number (%)[b])					
			Acinetobacter spp.	*E. coli*	*Klebsiella* spp.	*Proteus* spp.	*S*. Typhi	*S*. Paratyphi
I	Aminopenicillins	Ampicillin	3(60.0%)	-	-	-	-	-
		Amoxicillin	3(60.0%)	74(65.5%)	6(42.9%)	1(33.3%)	11(64.7%)	0
II	Sulphonomides	Co-trimoxazole	2(40.0%)	22(19.5%)	0	1(33.3%)	0	0
III	Quinolones	Ciprofloxacin	3(60.0%)	6(5.3%)	0	1(33.3%)	3 (17.7%)	0
IV	Aminoglycosides	Gentamycin	4(80.0%)	15(13.3%)	0	1(33.3%)	4(23.5%)	0
		Amikacin	4(80.0%)	58(51.3%)	4(28.6%)	1(33.3%)	-	-
V	Miscellaneous	Nitrofurantoin	5(100.0%)	80(70.8%)	7(50.0%)	1(33.3%)	-	-
VI	Cephalosporins	Cefotaxime	2(40.0%)	45(38.8%)	5(35.7%)	1(33.3%)	6(54.6%)	0
		Ceftriaxone	2(40.0%)	58(51.3%)	6(42.9%)	1(33.3%)	6(54.6%)	0
		Cefixime	-	-	-	-	10(58.8%)	-
VII	Tetracycline	Chloramphenicol	-	-	-	-	3(17.6%)	0

[a] Percent calculated:

(Total resistant of spp. / total isolates spp.)*100%

[b] Resistivity in the given table of a particular group includes the number of the isolates either resistance to single type of drug or both.

Table 4 shows the occurrence of the Multidrug resistant organisms among the clinical isolates. Multidrug in this case was categorized for those isolates which are resistant to one or more classes of antimicrobial agents[1]. The total of 83/159 (%) of gram negatives were found to be MDR.

Table 4: Occurrence of MDRO in clinical isolates

Sample	*Acinetobacter* spp.	*E. coli*	*Klebsiella* spp.	*S*. Typhi	Total
Urine	2	66	6	-	74(89.2%)
Pus	2	2	0	-	4 (5.1%)
Blood	-	-	-	5	5(6.0%)
Total	4	68	6		83 (100%)

DISCUSSION

The occurrence of MDR among gram negatives in Nepal has been reported in different hospitals. Study done in B.P.Koirala Institute of Health and Sciences among *Acinetobacter* spp. isolated from various clinical samples 99.2% were resistant to cefotaxime, 98.4% were resistant to ceftazidime, 95.9% were resistant to tobramycin, 96.7% were resistant to ciprofloxacin among 123 resistant strains. [16] In the another study done among the respiratory pathogens showed that more that 50% of gram negative isolates were resistant to ciprofloxacin, gentamicin, ampicillin and cephalexin.[17] Similarly on analyzing MSU samples 27.5% isolates of *E. coli* and *K. oxytoca* among

371 samples were confirmed for production of ESBLs. [18] In a study done at Kathmandu Model Hospital, it was found that the predominant bacteria causing UTI were the gram negative isolates constituting 88.2% among them 67.9% were MDR strains whereas gram positive bacteria constituted only 11.8% out of which 38.9% were MDR strains.[19] In the research done in TUTH total of 541 isolates of *S. enterica* serotypes Typhi (47%) and Paratyphi A (53%) were grown. Twenty-eight isolates (5%) of *S. enterica* were resistant to two or more antibiotics (MDR isolates), with a greater prevalence among serotype Paratyphi A (7%). All ESBLs producers (three isolates) were serotype Paratyphi A. [20] Regarding *P. aeruginosa* one of the study on MDR/ESBLs producing strains among respiratory pathogens more than 50% of total isolates of *P. aeruginosa* were resistant to ciprofloxacin, gentamycin, ceftazidime and ceftriaxone.[21]

The AST of gram negative isolates included in this study showed higher sensitivity towards ciprofloxacin, gentamycin and co-trimoxazole and higher resistance against amoxicillin, amikacin, nitrofurantoin followed by cefotaxime and ceftriaxone. Similar type of results was reported in Jordon in 2005 they reported excellent activity of ciprofloxacin against all gram-negative isolates. [22] In the study of Istanbul high rate of resistance was observed among the gram negative isolates against all antibiotics studied [23] and so was with this study. Such variations in the antimicrobial sensitivity pattern among different studies may be due to the variation in duration and dose of antibiotics used, spectrum of antibiotics used, and differing antibiotic policies among different hospitals.

The rise in resistance of organisms to amkacin and amoxicillin in this study may be due to increased consumption aminoglycosides and aminopenicillins in the hospital. In present study Enterobacteriaceae (*E. coli and Klebsiella* spp.) isolates with decreased susceptibility to cefotaxime and ceftriazone were defined as extended spectrum β-lactamase (ESBL) phenotypes. Similarly, *Acinetobacter* spp. in this study showed the high MDR nature showing resistance to more than 3 groups of antibiotics among aminopenicillins aminoglycosides, sulphonamides, quinolones, cephalosporins and other miscellaneous drug. Thus this study highlights the trends of multidrug resistance among gram negative isolates, therefore indicating an alarm of threat of emergence of drug resistant pathogens

During the last several decades, the prevalence of MDRO in hospitals and medical centers has increased steadily. Gram negative bacteria resistant to third generation cephalosporin fluoroquinolones, carbapenems, and aminoglycosides also have increased in prevalence. In 1997, The Senetry Antimicrobial Surveillance Program found that among *K. pneumoniae* strains isolated in the US, resistance rates to ceftazidime and other third-generation cephalosporin were 6.6%, 9.7%, 5.4%, and 3.6% for bloodstream, pneumonia, wound and urinary tract infections respectively. [24]

A 3-month survey of 15 Brooklyn hospitals in 1999 showed that 53% *of A. baumannii* strains were resistance to carbapenems and 24% of *P. aeruginosa* strains were resistant to imipenem. [25]

In 2003, 20.6% of all *K. pneumoniae* isolates from NNIS ICUs were MDR (NNIS, 2003). Similarly, between 1999 and 2003, *P. aeruginosa* resistance to fluoroquinolone antibiotics increased from 23% to 29.5% in NNIS ICUs. [26]

Extended spectrum β-lactamases (ESBLs) were detected in 33 (94.3%) isolates among the 35 isolates from the clinical sample from Taiwan. [27] Similarly the occurrence of ESBLs producing *E. coli* has been reported from New Dehli in GB Pant hospital where of the of 2,870 blood samples of suspected cases of septicemia between January and December 2009 Forty-one (70.7%) *K. pneumoniae* isolates and ten (41.7%) *E. coli* isolates were ESBLs. [28] In the study conducted in US 95% of the *K. pneumoniae* isolates showed resistance to at least one of the three third generation cephalosporin [3GC (ceftazidime, cefotaxime, ceftriaxone)] used for the study.[29] 87% of the *K. pneumoniae* isolates showed resistance to all the three third generation cephalosporin antibiotics and this resistance to all the three 3GC was found to coexist with resistance to other antibiotics. [30]

The prevention of NI demands a thorough knowledge of the infection rates and of the source, type and nature of invading microorganisms along with the risk factors associated with infection.[31] In developed countries many surveys and control programs are implemented so as to prevent transmission of pathogens from hospital environment to the patients. [32-35] On the other hand, in the context of resource poor countries like Nepal, there are scarce researches to identify the nosocomial pathogens that have hospital (exogenous) origin and are responsible for disease causation in immunocompromised patients. This cross sectional study therefore focuses on the MDR gram-negative organisms isolated from clinical sample during same period. But it was beyond the scope of this study to determine whether the isolates from clinical samples played role in causing the NI or not. However current study examined the pattern of the antibiotic resistance of the total clinical isolates on the basis of resistivity pattern shown in Kirby Bauer disc diffusion technique. Though the study was carried out in a single hospital, nonetheless this study will arrest the attention of all hospitals' management committee in making appropriate surveys and investigations to control development of resistant strain and infections from these.

CONCLUSION

In conclusion, although the hospital under investigation has been providing both long term and short term treatment to almost all types of cases for more than last two decades but had no report of analysis of prevalence of MDRO. So the result of this study may be an evidence for the need of management of NI and control development of MDR strain.

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