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Original Article

ANTIBIOTIC RESISTANCE PATTERNS OF GRAM NEGATIVE ISOLATES IN A TERTIARY CARE HOSPITAL OF NEPAL

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ABSTRACT

Introduction: Antimicrobial resistance in the current centuries has been the cause of lack of treatment of even common diseases. Although there are various antibiotics that can be used to combat Gram negative infection, resistant strains have subsequently emerged giving rise to life threatening superbugs.

Objective: The aim of the present study is to establish the incidence of gram negative bacteria in clinical specimens and their antibiotic sensitivity pattern.

Methods: In the present study Gram negative pathogens in clinical specimens were considered to determine prevailing antibiotic sensitivity pattern. Isolates were identified and screened for 13 different antibiotics by Kirby-Bauer disc diffusion method.

Results: A total of 1673 clinical samples were studied in a 8 months period. Of the total samples, 531 (31.74%) showed significant growth with 344 gram negative isolates. E. coli (179/344), Pseudomonas (87/344), Proteus (31/344), Klebsiella (28/344), Salmonella (12/344) and Enterobacter (7/344) were isolated. E coli was found to be most sensitive to cephalosporins and tetracycline and most resistant to quinolones, fluroquinolones and sulphonamides. For Pseudomonas, Amikacin and Ampicillin were most effective and Nalidixic acid was least effective. 114 of the isolates were found to be Multi Drug Resistant with E. coli 45.25% (81/179), Klebsiella 50% (14/28), Proteus 35.48% (11/31), Pseudomonas 2.3% (2/87), Salmonella 8.3% (1/12) and Enterobacter 71.42% (5/7).

Conclusion: Among all the tested antibiotics cephalosporins and fluroquinolones were most effective in compare to others.

Keywords: Gram Negative, Multi Drug Resistant, Antimicrobial Resistance

INTRODUCTION

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Antimicrobial resistance is a well-known clinical and public health problem [1].

This is an emerging public health problem, especially in hospitals of the newly industrialized countries of Asia and the Pacific [2, 3].

Microorganisms have developed the ability to make altered receptors for antimicrobial agents, have prevented agents from reaching their receptors within the bacterial cell, and now have enzymes to destroy antibiotics and have resistant metabolic pathways. Resistance based on decreased entry of drugs has been found for penicillins, cephalosporins, aminoglycosides and tetracyclines in the Enterobacteriaceae and Pseudomonas aeruginosa. Beta-lactamase resistance has increased significantly being encountered in Neisseria, Haemophilus, Enterobacteriaceae and Pseudomonas species [4,3].

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms [5]. 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 [6,7,8,9]

Knowledge of epidemiological and antimicrobial susceptibility pattern of common pathogens in a given area helps to inform the choice of antibiotics[10]. We report the pattern of Gram negative bacterial isolates in clinical samples and determine their antibiotic sensitivity pattern with special interest to MDR.

Methods and Methodology

In this study all the samples received in our hospital for microbiological analysis were taken into consideration. Samples were cultured in the respective media for the isolation of causative agents and identification of the organisms 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 (11,12,13). Antibiotic resistivity were analysed based on the Kirby disc diffusion method. Antibiotic disc Norfloxacin (10 mcg/disc), Ofloxacin (5 mcg/disc), Nalidixic Acid (30 mcg/disc), Ciprofloxacin (5 mcg/disc), Amikacin (30 mcg/disc) Amoxycillin (30 mcg/disc), Ampicillin (10 mcg/dis), Cloxacillin (10 mcg/disc), Tetracycline (30 mcg/disc), Cotrimoxazole (25 mcg/disc), Cephalexin ((30 mcg/disc), ceftriazone (30 mcg/disc) and chloramphenicol (30 mcg) were used. Interpretation of the results was done using the zone of inhibition sizes provided by the antibiotic disc manufacturer (Hi-Media).

RESULTS

Total of 1673 of the clinical samples received in a period of 8 months were considered in this study. 531 (31.73%) samples were found to be positive in culture and 344 (20.5%) of the samples were gram negative organisms. The significant growth of the isolates from different clinical samples is listed in the table 1.

Total of 6 different gram negative isolates were identified, distribution of the isolates in the sample is listed in table 2. Antibiotic resistivity pattern of 7 different antibiotic group were done in study, in which most effective drug compared to other was found to be cephalosporins with an average of approx. 5% resistivity in all organisms. Details in table 3.

33.14% of the isolated gram negative organisms were found to be multidrug resistant. MDR were classified as organisms resistant to two or more antibiotics (14). Although the frequency of organisms varies significantly, highest number of MDR was found to be *Enterobacter* followed be *Klebsiella*, *E.coli*, *Proteus, Pseudomonas*, *Salmonella.* (Table 4)

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

S. No.	Sample	Total	Significant growth	Gram Negative	in %
1	Urine	768	257	205	79.76
2	Blood	531	133	96	72.18
3	Pus	117	85	26	30.58
4	Sputum	101	35	5	14.29
5	Others	156	21	12	57.14
	Total	1673	531	344	64.78

Table 2: Distribution of bacterial isolates in different clinical samples

S.No.	Organism	Sample					Total
		Urine	Blood	Pus	Sputum	Others	
1.	E. coli	146	12	16	0	5	179
2.	Klebsiella	22	0	0	5	1	28
3.	Proteus	26	1	2	0	2	31
4.	Pseudomonas	4	71	8	0	4	87
5.	Salmonella	0	12	0	0	0	12
6.	Enterobacter	7	0	0	0	0	7
	Total	205	96	26	5	12	344

Table 3: Antibiotic Resistance Pattern of Gram Negative Isolates

Group	Antibiotics	Organisms (n (%))					
		E. coli	Klebsiella	Proteus	Pseudomonas	Salmonella	Enterobacter
Quinolones / Fluoroquilones	Norfloxacin (Nx)	67(44.96)	10(35.71)	5(16.13)	7(8.04)	1(8.33)	5(71.42)
	Ofloxacin (OF)	40(26.8)	2(7.14)	2(6.45)	2(2.30)	0	0
	Nalidixic Acid (NA)	43(28.85)	8(28.57)	8(25.81)	26(29.89)	3	0
	Ciprofloxacin (Cip)	65(43.62)	8 (28.57)	7(22.58)	2(2.3)	0	5(71.42)
Aminoglycosides	Amikacin (Ak)	10(6.71)	5 (17.85)	2(6.45)	1(1.15)	1(8.33)	1(14.29)
Penicillins	Amoxycillin (Amx)	47(31.54)	6 (21.42)	6(19.35)	3(3.45)	0	0
	Ampicillin (Amp)	10(6.71)	0	0	1(1.15)	0	0
	Cloxacillin (Cox)	41 (27.52)	8(28.57)	9(29.03)	9(10.34)	0	2(28.57)
Tetracycline	Tetracycline (TE)	5(3.36)	2(7.14)	2(6.45)	0	1(8.33)	0
Sulphonomides	Cotrimoxazole (CoT)	65(43.62)	12(42.86)	14(45.16)	5(5.74)	1(8.33)	3(42.86)
Cephalosporins	Cephalexin (Cp)	7(4.69)	4(14.29)	0	3(3.45)	0	0
	Ceftriazone (CTR)	8(5.37)	1(3.57)	1(3.22)	2(2.3)	0	0
Miscellaneous	Chloramphenicol (C)	12(8.05)	1(0.35)	3(9.68)	0	1(8.33)	0

Table 4: Occurrence of Multi drug resistant organisms

Organisms	Total	MDR (%)
E. coli	179	81 (45.25)
Klebsiella	28	14 (50.0)
Proteus	31	11(35.48)
Pseudomonas	87	2(2.30)
Salmonella	12	1(8.30)
Enterobacter	7	5 (71.42)
Total	344	114 (33.14)

DISCUSSION

In the study 33.14 % percent of the isolates were found to be MDR. Of the total organisms E. coli were found to be most MDR however distribution of the isolates was not equal. Cases of occurrence of MDR isolates in Nepal has been reported in different diseases. In the similar study conducted in tertiary hospital of capital in Nepal 52.2% of the gram negative isolates were found to be MDR [15]. In study of nosocomial infection in lower respiratory among the patients of TUTH 76.2% MDR in Pseudomonas spp., 97.2% MDR in Acinetobacter, 100% in Klebsiella spp were reported to be MDR [16]. On study of uropathogens the most prevailing MDR organism was found to be E. coli followed by Citrobacter. In the same study 55.2% of subsets of MDR E. coli was reported to be Extended Spectrum Beta Lactamase producers. The resistance pattern was reported to be alarmingly high for Amoxycillin, Cotrimoxazole, Fluoroquinolones and third generation cephalosporin. On contrary, in our study Fluroquinolones, Cotrimoxazole and Penicillin respectively were found to be most resistant antibiotics [17].

These MDR bacteria acts as a source of nosocomial infections in hospital, several of which are often resistant to many antimicrobials because of the selective pressure due to extensive use of broad-spectrum antibiotics in patients [18,19,20]. In this study high level of resistance was seen against quinolones/fluroquinolones which is in contrast to a Turkish and

Iranian studies where very high resistance was seen against cephalosporins and aminoglycosides [21, 22]. Gram-negatives were mostly resistant to aminoglycosides, in our study also aminoglycosides resistant organisms were reported [23].

In our study MDR is high in *Klebsiella* (50%), *E. coli* (45.25%) and low in Pseudomonas (2.3%) which is in contrast to a study in Canada where MDR is common on Pseudomonas and uncommon in *E. coli* and *Klebsiella* [24]. In case of Klebsiella infection In 1997, the SENTRY Antimicrobial Surveillance Program found that, among *K pneumoniae* strains isolated in the United States, the resistance rates to ceftazidime (as well as ceftriaxone and cefotaxime) were 6.6%, 9.7%, 5.4%, and 3.6% for bloodstream, pneumonia, wound, and urinary tract infections, respectively. Substantially higher resistance rates were noted in some of the individual hospitals enrolled in the study, and resistance rates of 30 to 50% were observed in the Latin American institutions studied [25]. Also, resistance of *K pneumoniae* strains to ceftriaxone has been reported in epidemics [26, 27]. Alternative antimicrobials that may be considered for use in patients with infections due to ESBL-producing strains of *K pneumoniae* include β-lactamase inhibitor combinations, such as piperacillin/tazobactam, and carbapenems [28]. Cross-resistance may limit the value of aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole in these types of infections. Fluoroquinolone resistance is also increasing among these ESBL strains. In the 1997 SENTRY Antimicrobial Surveillance Program, 2.1% of *E coli*, 13.3% of *P aeruginosa*, 24.1% of Acinetobacter, and 48.5% of *S maltophilia* isolates obtained in the United States were resistant to ciprofloxacin [29]. Furthermore, in the subgroup of patients with lower-respiratory-tract infections, 1.1% of *E coli*, 16.3% of *P aeruginosa*, 37.7% of Acinetobacter, and 42.7% of *S maltophilia* isolates from the United States and Canada were resistant to ciprofloxacin. The rates of ciprofloxacin resistance are substantially higher in Latin America. In addition, the 1998 SENTRY Antimicrobial Surveillance Program data demonstrate that an even higher percentage of *P aeruginosa* strains isolated from lower-respiratory-tract infections are resistant to the newer fluoroquinolones [30].

ESBL producing organisms are reported in many clinical cases in Nepal. These organisms are reported to resistant to third generation cephalosporins. In the study from tertiary hospital 92.6% MDR organsims isolated from the pyogenic infection were found to be ESBL producing [31]. In the present study though no ESBL production was phenotypically characterized but the third generation cephalosporin resistant organisms were found 4.69% and 5.37% of E. coli, 14.29 and 3.57% of Klebsiella, 3.45% and 2.3 % of Pseudomonas were found resistant to cephalexin and ceftriazone respectively. Cases of K. pneumonia and K. oxytoca producing extended spectrum β- lactamases and resistant to Floroquinolones, Aminoglycosides, Tetracycline and Cotrimoxazole has been reported in Nepal [32]. Similarly gram negative isolates resistant to third generation cephalosporins is reported in many other study done in Nepal [33]. Resistance to fluoroquinolones, co-trimoxazole, and trimethoprim is frequently observed among ESBL producers [34, 35]. Similar cases of resistance in both 3rd generation cephalosporins and sulphonamides were reported in our study.

Gram-negative infections were responsible for more severe infections and case fatality [36]. Severity of the cases increased by drug-resistant pathogens in hospitalized patients with serious infections such as pneumonia, urinary tract infections (UTI), skin and skin-structure infections, and primary or secondary bacteremia which is generally ascribed to the widespread use of antimicrobial agents [37]. In a recent report the Infectious Diseases Society of America specifically addressed three categories of MDR gram-negative bacilli, namely, extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp., MDR *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter* spp. [39]. Moreover, there are now a growing number of reports of cases of infections caused by gram-negative organisms for which no adequate therapeutic options exist [38]. This return to the preantibiotic era has become a reality in many parts of the world [40, 41 ad 42]. Among the species *E. coli* and *K. pneumoniae*, a worrisome trend during the last two decades has been the development of resistance to extended-spectrum cephalosporins, e.g., cefotaxime, ceftazidime, and ceftriaxone [43].

For the prevention of nosocomial infections 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 is the starting point [44]. Cases of resistant gram negative organisms has been reported in different surveys and control programs are implemented so as to prevent transmission of pathogens from hospital environment to the patients. However the impact of the interventions are not seen as desirable, emerging of the resistance in superbugs is still prevailing. Even as simple disease causing commensal flora has been the reason for the significant morbidity in most of the cases in Nepal. This cross sectional study therefore focuses providing the current trend of MDR gram-negative organisms among clinical sample so as to keep track of the resistivity that may rise is future and most important to know the massive use of the particular antibiotics and also their misuse so that measures could be taken to prevent severe consequences. 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. And thus we recommend to imply more sensitive techniques to identify the superbugs.

CONCLUSION

In conclusion, in Nepal trend of antibiotic resistance is increasing. Hospital under study, provides both long term and short term treatment to almost all types of cases. And this is the first incidence of the study of the resistivity pattern among the gram negative isolates in its long history of establishment. So the results of this study may be an evidence for the need of management of use of antibiotics.

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