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Original Article

Antimicrobial Resistance Patterns in Clinical Isolates of Enterobacteriaceae

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ABSTRACT

Introduction: Multidrug resistance among Enterobacteriaceae is in increasing trend these days. The objective of this study was to determine the antibiogram of clinical isolates of Enterobacteriaceae with special reference to multidrug resistance and extended spectrum beta-lactamases production.

Materials and Methods: A descriptive cross sectional study was conducted over a period of six months (February -July, 2017) in the microbiology laboratory of Nepal Medical College Teaching Hospital, Kathmandu, Nepal. A total of 936 bacterial isolates of Enterobacteriaceae from clinical specimens were processed for antimicrobial susceptibility testing and screened for multidrug resistance. ESBL production was detected among potential isolates by combination disk diffusion test.

Results: The rate of multidrug resistance and extended spectrum beta-lactamases production was 54.2% and 23.8% respectively. Of the total ESBL producers 92.4% were multidrug resistance. The rate of multidrug resistance and extended spectrum beta-lactamasesproduction were higher in organisms isolated from clinical samples collected from inpatients. High rate of multidrug resistance and extended spectrum beta-lactamases production was seen in *E. coli* (54.4% & 27.7%), *Klebsiella* spp. (67.1% & 28.2%) and *Citrobacter* spp. (70.3% & 10.9%). The antimicrobial resistance rate was highest against ampicillin (76.7%) followed by cefixime (54. 0%), ceftazidime (51.5%), ceftriaxone (51.0%), cotrimoxazole (48.7%), ciprofloxacin (43.9%) and ofloxacin (41.1%).

Conclusions: Multidrug resistance is common among Enterobacteriaceae. These bacteria have high rate of resistance against commonly used groups of antibiotics like cephalosporins and quinolones. Continuous monitoring, surveillance of antimicrobial resistance, proper infection control and practices are important to combat with these issues.

Key words: Enterobacteriaceae; ESBL; MDR

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INTRODUCTION

Enterobacteriaceae, a large, diverse group of facultative Gram-negative rods, are common pathogens of healthcare and community-associated infections worldwide.[1] Emergence of multidrug resistance (MDR) in Enterobacteriaceae is a major public health threat which poses a great challenge to combat infections.[2] Infections by extended spectrum β- lactamase (ESBL) producing Enterobacteriaceae are the most important among the causes of infections in the community and hospital in the recent years and are in rising trends.[3,4]

ESBLs are the mutant forms of β-lactamases enzymes encoded by plasmid genes and mediate resistance to extended spectrum cephalosporins and monobactams but do not affect cephamycins (e.g. cefoxitin and cefotetan) or carbapenems and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam. ESBL producing organisms are usually MDR as the plasmids carrying ESBL genes can carry resistant genes to other antibiotics like aminoglycosides, sulfonamides etc. These plasmid borne genes can be easily spread from one organism to another as they are easily transferable. Similarly widespread use of antibiotics like third generation cephalosporins is believed to be the major cause of the mutation in these genes.[4-7] Therefore

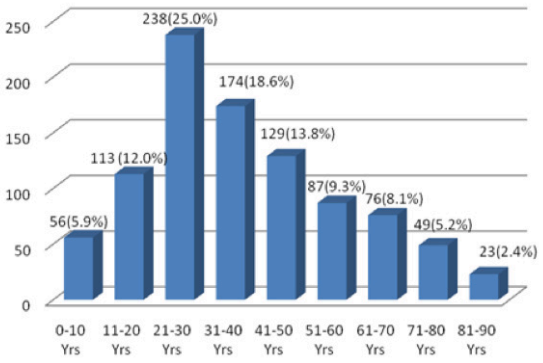

Figure1: Distribution of clinical isolates of Enterobacteriaceae according to the age of the patients, (n=936)

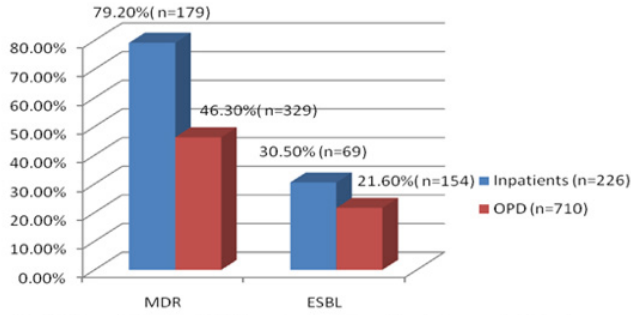

Figure 2: Rate of MDR and ESBL producing Enterobacteriaceae isolates in clinical samples from in-patients and OPD.

widespread and inappropriate use of antibiotics results emergence of MDR as well as ESBL producing organisms and are more common to spread in hospitals and ICU settings.[8] However several studies have shown their presence in community set up too.[9]

According to the 2013 report of the Centers for Disease Control and Prevention, ESBL producing Enterobacteriaceae were classified as a serious threat and are in constant rise in infections.[2] However, their prevalence rate varies with different geographical area. In Nepal, varying rate of ESBL producing organisms has been reported from different regions.[10-15] In this era of widespread resistance among both community and nosocomial pathogens, improved knowledge of local and regional epidemiology and susceptibility patterns is crucial in order to optimize empiric antibiotic treatment strategies. Thus this study was designed to know the patterns of antimicrobial resistance along with ESBL production among Enterobacteriaceae isolated from the different clinical specimens in our set up.

MATERALS AND METHODS

A descriptive cross sectional study was conducted over a period of six months (February–July 2017) in the Microbiology laboratory of Nepal Medical College Teaching Hospital (NMCTH), Kathmandu, Nepal. This research was approved by the Research and Institutional review committee (IRC) of Nepal Medical College Teaching Hospital, Kathmandu, Nepal. Letter of approval was obtained after submitting and presenting the proposal to the committee. Verbal consent was taken to all patients to include them as a sample source in the study. The study was done in 936 non-repeated bacterial isolates of Enterobacteriaceae from clinical specimens (pus, blood, urine, sputum and body fluids) from patients attending NMCTH.

Isolation and identification:

All the clinical samples received in the Microbiology laboratory for culture and sensitivity were processed as a routine diagnostic process by standard microbiological techniques.[16] In brief, the specimens were inoculated in culture plates (urine in CLED media, pus in blood agar and Mac-Conkey agar, sputum and body fluids in blood agar, Mac-Conkey agar and chocolate agar). All inoculated plates were incubated at 37^0C for 24 hours aerobically. All received blood culture bottles were, incubated at 37^0C and after 24 hours, sub-cultured in blood agar and Mac conkey agar every alternate day for seven days. Bacterial isolates of family Enterobacteriaceae were then identified further by studying colony characters, gram stain and biochemical tests.

Antimicrobial susceptibility Test

The antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method in Mueller Hinton agar (MHA) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines[17] by using the following commercially available antimicrobial discs from Hi-media, Laboratories, Mumbai, India. Ampicillin (10µg), ceftazidime (30µg), ceftriaxone (30µg), cefixime (30µg), amikacin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25µg /23.75µg), imipenem (10µg), meropenem (10µg), tigecycline (15 µg), piperacillin/tazobactam (100µg/10 µg. For urinary isolates, nitrofurantoin (300µg) was also tested.

Screening of MDR and Potential ESBL Producers: In this study, the isolates that are resistant to at least one agent of three different classes of commonly used antimicrobial agents, were regarded as MDR.[18] The bacterial isolates with zone of inhibition (ZOI) ≤25mm for ceftriaxone, ≤22mm for ceftazidime, and/or ≤27mm for cefotaxime were considered as a potential ESBL producer as recommended by CLSI .[17]

Phenotypic Confirmation of ESBL: Isolates that were considered as potential ESBL producers by initial screening were emulsified in nutrient broth to adjust the inoculum density equal to that of 0.5 McFarland turbidity standards. Combination disk test (CDT), as recommended by the CLSI, was performed in all isolates presumed to be ESBL producers. In this test, ceftazidime (30) disk alone and in combination with clavulanic acid (ceftazidime + clavulanicacid, 30/10µg) disk were applied onto a plate of MHA with the test strain and then incubated in ambient air for 18 hours of incubation at 37^0C. Isolate that showed increase of ≥ 5 mm in the zone of inhibition of the combination disks in comparison to that of the ceftazidime disk alone was considered as ESBL producer.[17]

RESULTS

A total of 10,676 clinical specimens (urine -5580, blood- 2276, sputum-1288, pus-1153 and body fluids-379) from both inpatients and outpatients of all age groups received for aerobic bacterial culture and antimicrobial susceptibility testing at NMCTH were included in the study. Of the total specimens processed 1900 clinical samples showed bacterial growth with growth positivity rate of 17.79 %. The prevalence rate of Enterobacteriaceae was 49.2% (n=936) among the total bacterial isolates and 8.76% among the total clinical specimen processed. Of the total 936 (710 from OPD and 226 from IPD) bacterial isolates of family

Table 1: Prevalence of Enterobacteriaceae isolates from different clinical samples

Organisms	Urine	Blood	Pus	Sputum	Body fluids	Total
***Escherichia* coli**	**492**	**15**	**97**	**29**	**10**	**643**
***Klebsiella* spp.**	51	8	31	40	1	131
***Salmonella* Typhi**	-	44	-	-	-	44
***Citrobacter* spp.**	20	5	21	16	2	64
***Enterobacter* spp.**	18	1	3	12	-	34
***Proteus* spp.**	5	1	8	3	-	17
***Providencia* Spp.**	2	-	1	-	-	3
Total	**588**	**74**	**161**	**100**	**13**	**936**

Table 2: The rate of ESBL production and MDR among the Enterobacteriaceae isolates.

Organisms	MDR n (%)	ESBL n (%)
Escherichia **coli (n=643)**	350 (54.4)	174 (27.0)
Klebsiella **spp. (n=131)**	88 (67.1)	37 (28.2)
Salmonella **Typhi (n=44)**	00 (00)	00 (00)
Citrobacter **spp. (n=64)**	45 (70.3)	7 (10.9)
Enterobacter **spp. (n=34)**	18 (52.9)	5 (14.7)
Proteus **spp. (n=17)**	6 (35.2)	00 (00)
Providencia **spp. (n=3)**	1 (33.3)	00 (00)
Total n=936	**508**	**223**

Enterobacteriaceae 357 were from male and 579 were from female. The distribution of the isolates according to age group of patient is shown in figure 1. Prevalence of Enterobacteriaceae isolates among different clinical samples is shown in table 1.

Of the total Enterobacteriaceae isolates, 508 (54.2 %) were MDR and 223 (23.8%) were ESBL producers. Of the total ESBL producers 206 (92.4%) were MDR. Both the ESBL production and MDR was higher in Enterobacteriaceae isolates among the clinical samples collected from inpatients (fig.2). The rate of ESBL production and MDR among the Enterobacteriaceae isolates is shown in table 2. The antimicrobial resistance pattern of Enterobacteriaceae isolates is shown in table 3.

DISCUSSION

Drug resistance among the clinical isolates of Enterobacteriaceae has laid challenge by limiting their therapeutic options while treating the diseases. This emphasizes the demands on routine Clinical Microbiology laboratory to investigate the potential of MDR and ESBL production on every suspected isolates. This study has determined the frequency of different isolates of Enterobacteriaceae from clinical specimens and their antibiogram with special reference to MDR and ESBL production.

In this study 54.2% of the Enterobacteriaceae isolates were MDR which is similar to the study conducted in same city.[11] However this rate was higher as compared to the study conducted in other part of our country.[15] Even higher resistance rate than this study was reported in Africa by Leski et al[9] in 2016. There is more risk of MDR development by bacteria in hospitals and in the community in countries with limited resources where hygiene is poor, antibiotics are misused and absence of antimicrobial surveillance programme.[19-21] In this study higher rate of drug resistance could be due to lack of awareness and improper use of antibiotics in our set up. The prevalence of ESBL production among the Enterobacteriaceae in this study was 23.8% which is similar to the studies conducted in Nepal[10-15] and in India.[22] Test for ESBL production among bacterial isolates by CLSI recommended phenotypic method may not detect ESBL production among the ESBL producing isolates that co-produce Amp- C beta lactamase.[23] Since we did not detect the Amp- C beta lactamase coproducing ESBL isolates in this study, the prevalence rate could be higher than this in our set up. The prevalence rate of ESBL production among Enterobacteriaceae, ranging from 13.5% to 64.3% have been reported from different parts of the world.[7,9,25] This could be due to the variations in their antibiotic prescribing policies, awareness and health education that determines the ESBL production by organisms.[19-21] In the referral hospitals and ICUs the rate of drug resistance and production of ESBL is high among the bacteria because the referred patients from the peripheral primary care centers already are laden with varieties of inappropriate antibiotics which contribute increase in drug resistance. This explains the reason for higher prevalence rate of ESBL in this study since our study centre is one of the referral hospital.

MDR and ESBL producing bacteria are more prevalent in the hospital setting. In the recent days there are several evidences that they are emerging and spreading in the community as well.[15] This study showed higher rate of MDR and ESBL producing Eneterobacteriaceae isolates in clinical samples from inpatient which is similar to the above statement. But this study also showed 46.3% (329 of 710) and 21.6% (154 of 710) of the OPD isolates as MDR and ESBL producers respectively. This explores the significant presence of resistant organisms in the community and the need for preventive measures to be applied to limit their spread not only in hospital set up but also in the community as well.

MDR and ESBL production are commonly seen in *Klebsiella* spp. and *E. coli*[7,14,19] among the Enterobacteriaceae isolates. Similar pattern was seen in this study. Higher rate of MDR was seen in *Citrobacter* spp. however, it was not statistically significant. (P>0.05) Carbapenems, relatively expensive antibiotics are the choice of drug for ESBL producing organisms. This study showed almost 10 % of the isolates are resistant to carbapenems. Resistance to carbapenems in this study could be due to the production of carbapenemases and metallo B lactamases by the organism which were not looked for in this study.[4-6]

Among the second line drugs, resistance to piperacillin-tazobactam, tigecycline and amikacin were found to be low amongst the Enterobacteriaceae isolates in-vitro. But more than 50% of the isolates showed resistance against the commonly used antibiotics like cephalosporins and quinolones. Resistance to

Table 3: Resistance patterns of Enterobacteriaceae isolates to different antimicrobial agents.

Antibiotics used	E. coli (n=643) No (%)	Klebsiella spp. (n=131) No (%)	S. Typhi (n=44) No (%)	Citrobacter spp. (n=64) No (%)	Enterobacter spp.(n=34) No (%)	Proteus spp .(n=17) No (%)	Providencia spp. (n=3) No (%)	Total (n=936) No (%)
Ampicillin	482 (74.9)	131 (100)	9 (20.4)	59 (92.2)	32 (94.1)	10 (58.8)	2 (66.6)	718 (76.7)
Cefixime	341 (53.0)	89 (67.9)	4 (9.0)	50 (78.1)	21 (61.8)	4 (23.5)	1 (33.3)	506 (54.0)
Ceftazidime	328 (51.0)	88 (67.1)	4 (9.0)	43 (67.1)	19 (55.8)	3 (17.6)	1 (33.3)	482 (51.5)
Ceftriaxone	322 (50.0)	86 (65.6)	4 (9.0)	45 (70.3)	20 (58.8)	3 (17.6)	1 (33.3)	477 (51.0)
Piperacillin-Tazobactam	74 (11.5)	36 (27.4)	00 (00)	22 (34.3)	8 (23.5)	00 (00)	00 (00)	140 (14.9)
Amikacin	64 (9.9)	42 (32.0)	NT	31 (48.4)	12 (35.2)	1 (5.8)	00 (00)	145 (15.5)
Ciprofloxacin	291 (45.2)	66 (50.3)	8 (18.0)	28 (43.7)	15 (44.1)	3 (17.6)	00 (00)	411 (43.9)
Ofloxacin	266 (41.3)	66 (50.3)	8 (18.0)	28 (43.7)	14 (41.2)	3 (17.6)	00 (00)	385 (41.1)
Cotromoxazole	313 (48.6)	83 (63.3)	4 (9.0)	39 (60.9)	15 (44.1)	5 (29.4)	1 (33.3)	456 (48.7)
Imipenem	31 (4.8)	16 (12.2)	00 (00)	19 (29.6)	3 (8.8)	1 (5.8)	00 (00)	70 (7.5)
Meropenem	30 (4.7)	16 (12.2)	00 (00)	18 (28.1)	3 (8.8)	1 (5.8)	00 (00)	69 (7.4)
Tigecycline	2 (0.3)	3 (2.2)	00 (00)	00 (00)	00 (00)	4 (23.5)	00 (00)	10 (1.0)
Nitrofurantoin	40 (8.1)	17 (33.3)	NT	7 (35.0)	10 (55.5)	5 (100)	1 (50)	80 (13.6)
(no of urine isolates)	492	51		20	18	5	2	588

NT: Not tested.

β-lactams in Enterobacteriaceae is mainly due to the production of β-lactamases, which may be encoded either chromosomally or on plasmids. Several studies on Enterobacteriaceae isolates showed varieties of resistance rate against cephalosporins that ranges from 30 to 70%.[7,14,15] Increased resistance to cephalosporins among Enterobacteriaceae in this study could be due to the excessive use of cephalosporins in our set up. Quinolones like ciprofloxacin and ofloxacin also are other commonly prescribed antibiotics to treat bacterial infections. The resistance rate against these groups of antibiotics is also rising among the Enterobacteriaceae isolates.[7,9,14] Resistance to quinolones typically arises as a result of alterations in the target enzymes (DNA gyrases and topoisomerases IV) and of changes in drug entry and efflux. Wide spread use of fluoroquinolones has contributed to the rapid emergence of resistance worldwide.[4] Over the counter availability of all these antibiotics, open defecation system, lack of proper sewage system and lack of practice of isolation of patient infected with MDR organisms have resulted wide use of these antibiotics and spread of MDR organisms in our country that explains high rate of resistance against these groups of antibiotics.

CONCLUSIONS

Antimicrobial resistance among Enterobacteriaceae is a growing threat these days. Increased rate of resistance to the commonly used and relatively safer antibiotics like cephalosporins and quinolones explores the urgent need for alternatives to these groups of antibiotics. Low level of resistance against carbapenems and tigecycline were seen in this study. This does add little hope to fight against the infections by MDR bacteria; however these groups of antibiotics are the reserved drugs, expensive and have comparatively more adverse effects. It is therefore time to identify the causes and stop the spread of these resistant bacteria in hospital as well as in the community. Judicious selection of antimicrobial regimens, regular antimicrobial resistance surveillances are important to tackle these issues.

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