ORIGINAL ARTICLE

Occurrence of extended spectrum beta lactamase producing *Enterobacteriaceae* isolated from pus samples in a tertiary care hospital

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Aim: The aim of this study was to evaluate ooccurrence of extended spectrum beta lactamases (ESBL) producing *Enterobacteriaceae* isolated from pus samples in a tertiary care hospital. **Background:** *Enterobacteriaceae* are the common etiological agents that cause serious community and healthcare associated infections. Antibiotic resistance in these bacteria is evolving rapidly especially by production of ESBLs which has led to limited treatment options left for life threatening infections. Prevalence of ESBLs varies from region to region and time to time. Therefore, regular surveillance of local antibiogram is required to help clinicians in selecting appropriate empirical antibiotic therapy. **Materials and Methods:** In this cross-sectional study we have included one hundred isolates of *Escherichia coli* and *Klebsiella* spp. isolated from pus samples during 1 year. Antibiotic susceptibility testing was done by Modified Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines. ESBL testing was done by double disc synergy test. **Results:** Out of hundred isolates, 65 were *E. coli* and 35 were *Klebsiella* spp. The prevalence of ESBLs was found to be 45% which included 46.2% *E. coli*, 44.4% *Klebsiella pneumoniae* and 37.5% *Klebsiella oxytoca* isolates. Among study isolates, 34% isolates showed multi drug resistance. **Conclusions:** Alarming rise in antibiotic resistance is challenging worldwide, especially for developing countries. In concordance with recent studies, we have also found high prevalence of ESBLs which points towards a strong need for implementation of antimicrobial stewardship so as to curtail the further spread of antibiotic resistance.

KEY WORDS: Antibiotic resistance, extended spectrum beta lactamases, multidrug resistance

INTRODUCTION

The world is currently tackling with increasing antimicrobial resistance which is a major challenge in public health. Antimicrobial resistance among gram negative bacteria is more difficult to manage because of their ability to mutate and transmit resistance genes.[1] B lactam antibiotics are the mainstay of treatment against infections caused by gram negative bacteria. However, emergence of resistance to B lactam antibiotics, especially by production of extended spectrum beta lactamases (ESBLs) is challenging for clinicians. This leads to treatment failure, increasing morbidity and duration of illness which is a point of global concern.[2] ESBLs are a group of enzymes that cause hydrolysis of beta lactam antibiotics like Penicillin, Cephalosporins and Monobactams.[3] Since 1980s, *Enterobacteriaceae* especially *Klebsiella* spp. and *Escherichia coli* producing ESBLs have been identified to cause difficult to treat hospital-acquired infections as well as community-acquired infections such as wound infections, urinary tract infections, pneumonia etc.[4,5] Previous studies have reported that 25–50%

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of clinical isolates from *Enterobacteriaceae* family are ESBL producers.[6-9] Hence knowledge of local antibiogram is an important tool for clinicians in selecting empirical antimicrobial therapy and successful management of the patients. This study was conducted to elucidate ooccurrence of ESBL producing *Enterobacteriaceae* isolated from pus samples in a tertiary care hospital.

MATERIALS AND METHODS

The cross-sectional study was conducted in department of Microbiology in a tertiary care hospital from October 1, 2020 to September 30, 2021. One hundred isolates of *E. coli* and *Klebsiella spp.* from pus samples identified by standard microbiological procedures were included in the study. Modified Kirby Bauer's Disc Diffusion method was used to for antibiotic susceptibility testing and diameter of the zone of inhibition was interpreted as per Clinical and Laboratory Standards Institute guidelines.[10] Antibiotics such as Ceftazidime (30 µg), Cefazolin (30 µg), Cefaperazone/sulbactum (30/10 µg), Cefepime (30 µg), Cefuroxime (30 µg), Ampicillin (10 µg), Amoxyclav (20/10 µg), Amikacin (30 µg), Gentamicin (10 µg), Cefotaxime (30 µg), Aztreonam (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Imipenem (10 µg), Ertapenem (10 µg), Meropenam (10 µg), Tobramycin (10 µg), Tetracycline (30 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg) and Piperacillin tazobactam (100/10 µg) were used.

The isolates showing decreased zone diameter for third generation Cephalosporins (Ceftazidime <22 mm, Cefotaxime <27 mm, Ceftriaxone <25 mm) were considered as ESBL screening test positive. Further confirmation of ESBL production was done by double disc synergy test. In this test, on the inoculated Mueller-Hinton agar third-generation cephalosporins and amoxycillin-clavulanic acid discs were kept 20 mm apart, centre to centre.[11,12] Clear extension of the inhibition zone of cephalosporins toward amoxy-clav disc was considered as confirmation of ESBL production.

Data were entered into excel sheet and analysed by using SPSS 22 software. Descriptive data were represented as frequency and percentage.

RESULTS

All pus samples collected from different departments of our tertiary care hospital were processed and one hundred isolates were included in the study. The maximum number of isolates were recovered from the samples received from the surgery department (55) followed by orthopedics (23) [Figure 1]. *E. coli* (65) was the most commonly isolated organism followed by *Klebsiella pneumoniae* (27) and *Klebsiella oxytoca* (8).

Antibiotic susceptibility testing demonstrated that in *E. coli* isolates, maximum resistance was found against Cefazolin (62/65) followed by Cefuroxime (61/65), Piperacillin/Tazobactam (53/65), Cefotaxime (56) Ceftazidime (55) and Levofloxacin (50/65). Whereas least resistance was observed against Tobramycin (18/65) followed by Gentamycin (20/65) and Amikacin (27/65) [Figure 2].

In *Klebsiella* spp., the highest resistance was shown against Cefuroxime (33/35) followed by Ceftazidime (28/35), Levofloxacin (27/35), Piperacillin/Tazobactam (27/35) and Cefepime (24/35). Gentamicin (24/35), Amikacin (20/35) and Meropenem (19/35) were found to have better activity against the isolates [Figure 3].

In our study, 45% isolates were found as ESBL producers which included 46.2% *E. coli*, 44.4% *K. pneumoniae* and 37.5% *K. oxytoca* isolates. Among study isolates, 33.8% *E. coli*, 37% *K. pneumoniae* and 25% *K. oxytoca* were found to have multi drug resistance (MDR) [Table 1].

DISCUSSION

Emerging antibiotic resistance among *Enterobacteriaceae* especially *E. coli* and *Klebsiella* spp. is a matter of concern as they are capable of causing serious infections and posing a challenge to treatment. Moreover, resistance to cephalosporins has been strikingly on the rise worldwide leading to limited therapeutic options left for the treatment of infections caused by ESBL producing organisms.

We had included one hundred isolates from pus samples that comprised of 65 *E. coli*, 27 *K. pneumoniae* and 8 *K. oxytoca*. Majority of the isolates were recovered from pus samples

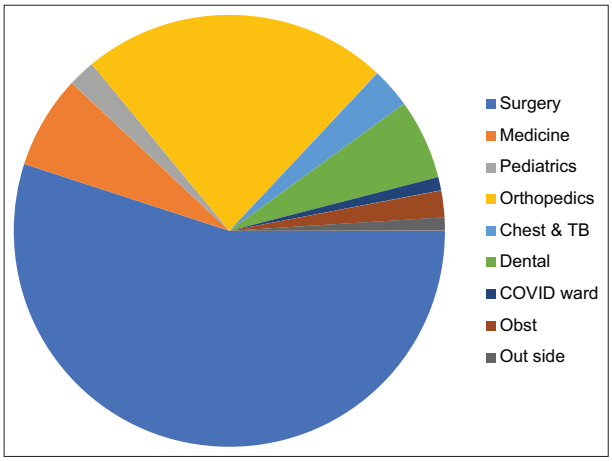

Figure 1: Distribution of samples received from different departments of the hospital

Table 1: ESBL production and multidrug resistance among study isolates

Organism	Isolates (*n*=100)	ESBL producers (*n*=45) (%)	MDR isolates (*n*=34) (%)
Escherichia coli	65	30 (66.7)	22 (64.7)
Klebsiella pneumoniae	27	12 (26.6)	10 (29.4)
Klebsiella oxytoca	8	3 (6.7)	2 (5.9)

ESBL: Extended spectrum beta lactamases, MDR: Multi drug resistance

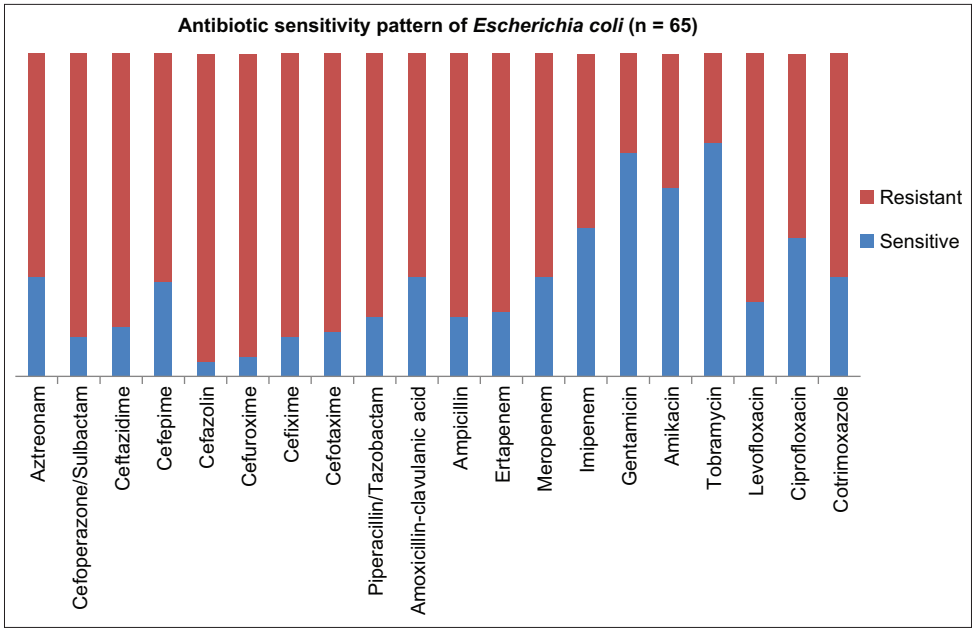

Figure 2: Antibiotic sensitivity pattern of *Escherichia coli* isolates

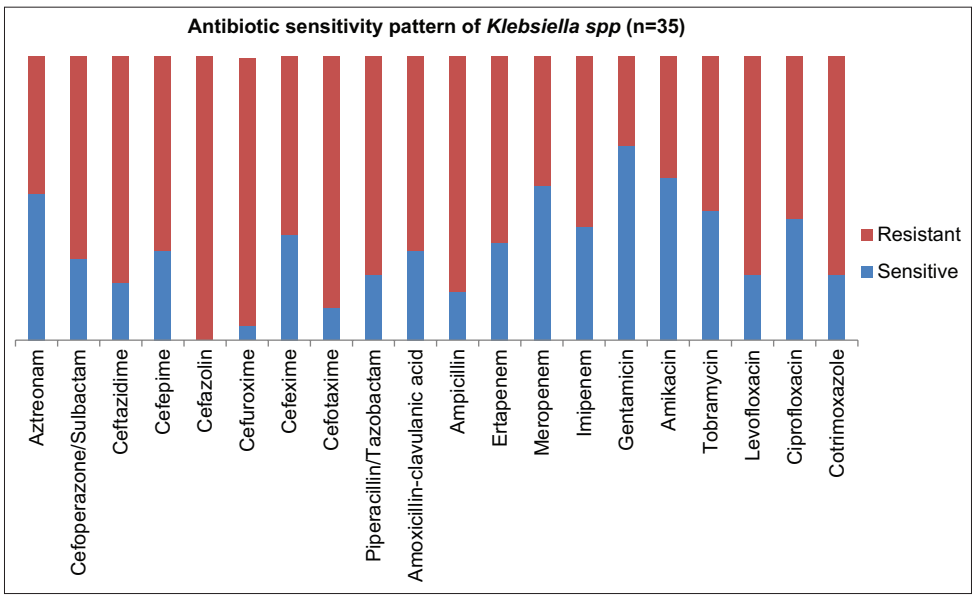

Figure 3: Antibiotic sensitivity pattern of *Klebsiella* spp. Isolates

received from the surgery (55%) and orthopaedic department (23%). Concordant higher isolation of gram-negative bacteria from surgical wards was observed by Trojan *et al*.[13] and Seni *et al*.[14]

The present study indicates high level of resistance to cephalosporins (80-90%), fluoroquinolones (76%) and amoxicillin-clavulanic acid (68%). This was also observed in studies conducted by Tekele *et al*.[8] and Trojan *et al*.[13] Because of broad-spectrum activity, β-lactams and fluoroquinolones remained the most commonly prescribed antibiotic for the treatment of gram-negative organisms over a decade. However, irrational and over the counter use of these antibiotics has led to evolution of resistance mechanism in organisms due to selection pressure. Alarmingly, sensitivity against carbapenems was found to be markedly reduced (45-60% resistance) which is the reserved drug for treatment of life-threatening infections. Similar decreased sensitivity towards carbapenams was reported by Ibrahim *et al*.,[7] Trojan *et al*.[13] Meanwhile we found higher sensitivity towards aminoglycosides (58-70%), similarly reported by Ibrahim *et al*.[7] and Trojan *et al*.,[13] making them a better treatment option in current scenario of growing antibiotic resistance.

In our study, prevalence of ESBL producers was 45% (n = 45) including 30 (66.6%) *E. coli*, 12 (26.6%) *K. pneumoniae* and 3 (6.6%) *K. oxytoca* isolates. Previous studies have observed variable rate of ESBL production in *E. coli* and *Klebsiella* spp. ranging from 30% to 70%.[6,9,15,16] This variation in prevalence of ESBL can be explained on the basis of differences in types of patient/sample selected and the trend of antibiotic usage in the various areas. In the current study, the predominant ESBLs

producing isolates were *E. coli* and this finding was similar to previous studies.[9,17] In contrast, studies conducted by Rawat *et al*.[11] and Seni *et al*.[14] reported higher prevalence of ESBLs in *Klebsiella* isolates.

We have also observed that almost one third (34%) isolates were multi drug resistant (resistant to three classes of antibiotics). Comparable results were found by Basak *et al*.[18] whereas higher prevalence of MDR organisms were reported by Tekele *et al*.[8] and Tewari *et al*.[19] Higher rates of MDR can be attributed to factors such as prolonged hospital stay, indiscriminate antibiotic use, use of *in-situ* catheters/tubes and patient co-morbidities. Moreover, MDR and ESBL prevalence goes hand in hand as ESBLs have capacity to spread by plasmids that usually carry resistance genes for different classes of antibiotics.

CONCLUSIONS

In the era of rising antibiotic resistance, patient treatment and outcome are being affected adversely. Rising health care cost due to limited and expensive antibiotic options left for the treatment of resistant organisms is challenging for developing countries like India. Our study also concludes high prevalence of ESBL and MDR organisms thus necessitating regular monitoring of local antibiogram to curtail misuse of broad-spectrum antibiotics. Regular update of hospital antibiotic policy and adherence to infection control and prevention measures can act as vital pillars in interruption of further spread of antimicrobial resistance.

REFERENCES

1. Nurul MA, Loo HK, Subramaniam G, Wong EH, Selvi P, Ho S, *et al*. Faecal prevalence of extended spectrum ß- lactamase (ESBL)-producing coliforms in a geriatric population and among haematology patients. Malaysian J Pathol 2005;27:75-81.
2. Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M, Sharifi SH. Genotypic versus Phenotypic methods to detect extended-spectrum beta-lactamases (ESBLs) in uropathogenic *Escherichia coli*. Ann Biol Res 2012;3:2454-8.
3. Sameena K, Lakshmi K, Chitralekha S, Aishwarya L Jr. Detection of extended spectrum beta lactamases in Escherichia coli isolates. Int J Pharm Biol Sci 2013;4:B1201-4.
4. Paterson DL, Bonomo RA. Extended-spectrum ß-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
5. Doi Y, Adams J, O'Keefe A, Quereshi Z, Ewan L, Paterson DL. Community acquired extended-spectrum b-lactamase producers, United States. Emerg Infect Dis Emerg Infect Dis 2007;13:1121-3.
6. Sandhiya R, Lakshmi PR, Esthermary S. Antibiotic susceptibility pattern and ESBL prevalence in *Escherichia coli* isolates from pus samples in a tertiary care hospital. Int J Pharm Pharm Sci 2015;7:263-4.
7. Ibrahim ME, Abbas M, Al-Shahrai AM, Elamin BK. Phenotypic characterization and antibiotic resistance patterns of extended-spectrum β-lactamase-and AmpC β-lactamase-producing gram-negative *Bacteria* in a referral hospital, Saudi Arabia. Can J Infect Dis Med Microbiol 2019;2019:9.
8. Tekele SG, Teklu DS, Tullu KD, Birru SK, Legese MH. Extended-spectrum betalactamase and AmpC beta-lactamases producing gram negative bacilli isolated from clinical specimens at international clinical laboratories, Addis Ababa, Ethiopia. PLoS One 2020;15:e0241984.
9. Assudani H, Gusani J. Prevalence of extended spectrum B lactamase producing *E. coli* and *Klebsiella* SPP isolated in a tertiary care hospital, Gujarat. Trop J Path Micro 2019;5:403-7.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. United States: CLSI Supplement M100, Clinical and Laboratory Standards Institute; 2020.
11. Rawat D, Nair D. Extended-spectrum ß-lactamases in Gram Negative *Bacteria*. J Global Infect Dis 2010;2:263-74.
12. Jarlier V, Nicolas MH, Fournier G, Philippon A. ESBLs conferring transferable resistance to newer-lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Rev Infect Dis 1988;10:867-78.
13. Trojan R, Razdan L, Singh N. Antibiotic Susceptibility pattern of bacterial isolates from pus samples in a tertiary care hospital of Punjab, India. Int J Microbiol 2016;2016:9302692.
14. Seni J, Najjuka CF, Kateete DP, Makobore P, Joloba ML, Kajumbula H, *et al*. Antimicrobial resistance in hospitalized surgical patients: A silently emerging public health concern in Uganda. BMC Res Notes 2013;6:298.
15. Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of extended spectrum beta lactamases (ESBL) producing gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. JCDR 2013;7:2173-77.
16. Wadekar MD, Swarooparani NB. ESBL producing Escherichia coli- its prevalence and antibiogram. Indian J Microbiol Res 2017;4:287-90.
17. Shashwati N, Kiran T, Dhanvijay AG. Study of Extended spectrum beta-lactamase producing *Enterobacteriaceae* and antibiotic co-resistance in a tertiary care teaching hospital. J Natl Sci Biol Med 2014;5:30.
18. Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: A study. J Pathog 2016;2016:5.
19. Tewari R, Mitra SD, Ganaie F, Venugopal N, Das S, Shome R, *et al*. Prevalence of extended spectrum beta-lactamase, AmpC beta-lactamase and metallo beta-lactamase mediated resistance in *Escherichia coli* from diagnostic and tertiary healthcare centers in South Bangalore, India. Int J Res Med Sci 2018;6:1308-13.