www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i6.125

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Study of antibiogram in family Enterobacteriaceae in a Teaching Hospital, Kolhapur, Maharashtra

Authors
Dr Vishwashanti S Vatkar[1], Dr Rashmi Penkar[2], Dr Saral Ghosh[3]
[1]Associate Professor, Microbiology Department, D Y Patil Medical College, Kolhapur
[2]Microbiologist, Apple Hospital, Kolhapur
[3]Associate Professor, Microbiology Department, D Y Patil Medical College, Kolhapur

Abstract
Infections due to gram negative bacilli (GNB) are more common and increasing antibiotic resistance amongst these infections is a worldwide problem. Enterobacteriaceae are frequently isolated from various clinical samples. Overuse or irrational use of antibiotics to treat these infections lead to develop antibiotic resistance, such multidrug resistant (MDR)-Enterobacteriaceae infections are difficult to treat. The present study was conducted to know the antibiogram of Enterobacteriaceae isolated from various clinical samples. This retrospective study was conducted in Dr D Y Patil Hospital & Research Centre, Kadamwadi, Kolhapur, Maharashtra, India over a period of one year (December 2016- December 2017). Identification and antibiotic sensitivity of Enterobacteriaceae were performed by standard microbiological procedures and VITEK II automated system. A total 235 Enterobacteriaceae GNB was isolated from various clinical samples. The most frequently isolated organism was Escherichia coli 45.5% followed by Klebsiella spp 31%, Citrobacterspp 11.9%, Proteusspp 9.3%, Serratiamarcescens 1.2% & Enterobacteraerogens 0.8% in the present study. Most of the organisms were isolated from urine sample 44% followed by pus 40%. Carbapenems, Tigecycline and Amikacin were effective against MDR-Enterobacteriaceae. Most effective antibiotics were Imipenem showed 97%, Meropenem 91%, Amikacine 91%, Gentamicin 83.4%, Piperacillin-tazobactum 79.5%, Tigecycline 97% and Ciprofloxacine 41,2% sensitivity. Most resistant antibiotics were Ampicillin showed 31.4%, Ceftazidime 25.5% and Ceftrixone 24.6% sensitivity in the present study.
Keywords: *Antibiogram, Enterobacteriaceae, Escherichia coli, Imipeneme.*

Introduction

Resistance to different antibiotics is a world-wide problem now days. Increasing antimicrobial resistance among Gram Negative Bacilli (GNB) is a major problem to treat infections in the community as well as in hospitalized patients [(1)]. A Multidrug resistant infection is difficult to treat and also increases the morbidity & mortality in critically ill patients, especially ICUs.

Irrational use of antibiotics by practitioners, lack of hospital antibiotic policy leads to increase antibiotic resistance. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to these life –

Saving drugs. Hospital antibiograms are commonly used to help guide antimicrobial treatment and help to detect and monitor pattern of antimicrobial resistance amongst the clinical isolates.

Gram negative bacilli are a large group of micro-organisms and amongst them Enterobacteriaceae are one of the most common bacteria isolated from various clinical samples.

Escherichia coli, Klebsiella spp, Proteus & Citrobacterspp are commonly isolated from clinical samples like urine, pus etc. other members such as *Serratiamarcescens* & *Enterobacter-aerogens* infections are also increasing [(2).]

ESBL producing GNB are clinically important because they causes multidrug resistant infections and very difficult to treat especially in patients in ICU, post-operative infections etc.[(3)]These enzymes are chromosomally or plasmid mediated so they show resistance to non-beta-lactam antibiotics like quinolones, aminoglycosides, chloramphenicol etc[(4)].

The present study was undertaken to know the antibiogram of GNB isolated from clinical samples at our teaching hospital.

Materials and Methods

The present study was carried out to know the antibiotic sensitivity pattern among GNB in family Enterobacteriaceae isolated from various clinical samples at Dr D Y Patil Hospital and Research Centre, Kadamwadi, Kolhapur, Maharashtra over a period of one year (December 2016 to December 2017)

Clinical samples like pus, urine, sputum, body fluids etc received in clinical microbiology laboratory of Dr D Y Patil Hospital & Research Centre, Kolhapur were plated on blood agar, MacConkey agar (Hi-Media, Mumbai) for primary isolation of organisms.

Preliminary identification of GNB was performed using conventional methods including: Gram-staining, culture characteristics, lactose fermentation, and oxidase test. Further identification to species level was performed using VITEK II (ID-GN 21-341 card) automated system (BioMerieux, France) and Minimum Inhibitory Concentration (MIC) of these GNB by using AST-N280 card according to manufacturer's instructions.

Antimicrobial susceptibility test (AST): Disc diffusion method

The susceptibility of the tested isolates was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Hi-Media, Mumbai) results were noted according to the Clinical and Laboratory Standard Institutes (CLSI) guidelines (CLSI, 2016)[(5)]. 0.5 McFarland standards used as inoculum, direct colony suspension for AST.

The commercial antibiotics discs (Hi – Media, Mumbai) used for Enterobacteriaceae were piperacillin-tazobactam (100/10 µg), ampicillin (10µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (10µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and nitrofurantoin (300 µg) for urine samples

Standard strains used were- *Escherichia coli*- 25922 as negative control and *Klebsiella pneumoniae*- 700603 as positive controls as quality control for identification and antibiotic susceptibility test of test strains.

Results

The total 235 GNB were isolated from different clinical samples.

Table 1: isolation of GNB from different clinical samples

organism	no	%age
Escherichia coli	107	45.5
Klebsiella spp	73	31
Citrobacterspp	28	11.9
Proteus spp	22	9.3
Serratiamarcescens	3	1.2
Enterobacteraerogens	2	0.8

Diagram 1: specimen wise distribution of GNB

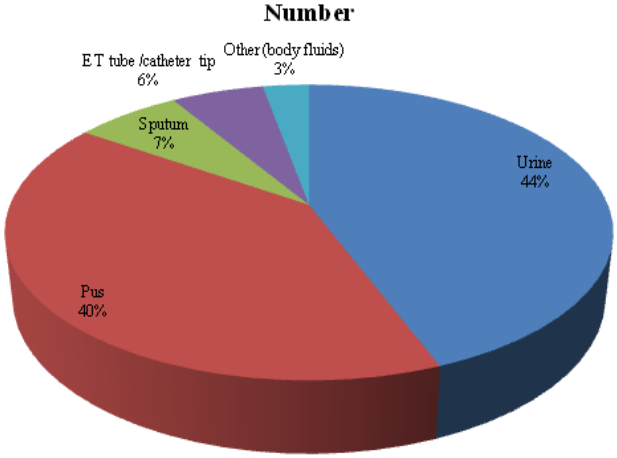


Table 2: distribution of organisms in samples

	Sample				
Organism	Urine	Pus	Sputum	Body fluids	ETtube/catheter tip
E coli (107)	57 (53.2%)	46 (42.8%)	2 (11.5%)	1 (0.9%)	1 (0.9%)
Klebsiella spp (73)	28 (38.3%)	26 (35.6%)	11 (15%)	2 (2.7%)	6 (8.2%)
Citrobacterspp (28)	9 (32.1%)	11 (39.2%)	3 (10.7%)	3 (10.7%)	2 (7.1%)
Proteus spp (22)	12 (54.5%)	9 (40.9%)	--	--	1 (4.5%)
Serraciamarcescens (3)	1 (33.3%)	1 (33.3%)	--	1 (33.3%)	--
Enterobacteraerogens (2)	--	2 (100%)	--	--	--

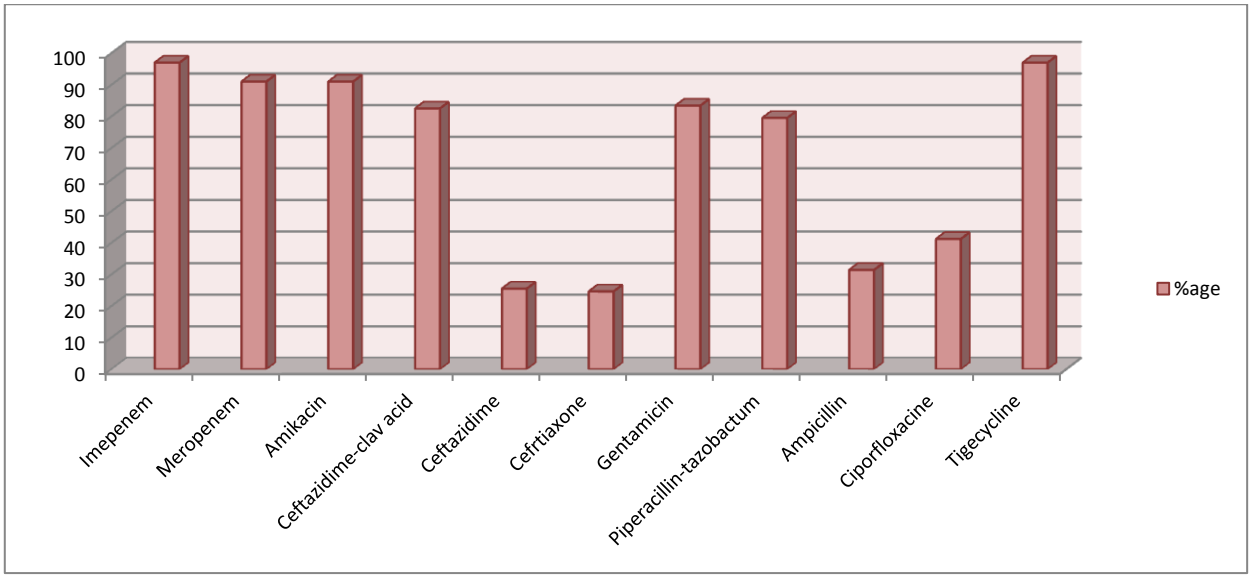
Table 3: Antibiotic sensitivity pattern of organisms

ANTIBIOTICS	**ORGANISM**					
	E coli (107)	***Klebspp*** (73)	***Citrob Spp*** (28)	***Proteus Spp*** (22)	***Serracia Spp*** (3)	***Enterob Spp*** (2)
Amikacin	95 (88.7%)	65 (89%)	25 (89.2%)	20 (90.9%)	3 (100%)	1 (50%)
Gentamicin	90 (84.7%)	60 (82.1%)	20 (71.4%)	18 (81.8%)	3 (100%)	1 (50%)
Caftezicime-clav acid	90 (84.7%)	58 (79.4%)	22 (78.5%)	7 (77.2%)	2 (66.6%)	1 (50%)
Ceftazidime	22 (20.5%)	20 (27.3%)	8 (28.5%)	8 (28.5%)	2 (66.6%)	1 (50%)
Ceftriaxone	25 (23.3%)	15 (20.5%)	7 (25%)	7 (31.8%)	2 96.6%)	1 (50%)
Imipenem	105 (98.3%)	69 (94.5%)	26 (92.8%)	20 (90.9%)	3 (100%)	2 (100%)
Meropenem	102 (95.3%)	65 (89%)	24 (85.7%)	15 (68.1%)	3 (100%)	2 (100%)
Piperacillin-tazobactum	85 (79.4%)	60 (82.1%)	20 (71.4%)	15 (68.1%)	3 (100%)	1 (50%)
Ampicillin	37 (34.5%)	21 (28.7%)	9 (32.1%)	5 (22.7%)	--	1 (50%)
Ciprofloxacine	62 (57.%)	14 (19.1%)	8 (28.5%)	8 (36.3%)	2 (66.6%)	2 (100%)
Tigecycline	105 (98.1%)	71 (97.2%)	24 (85.7%)	20 (90.9%)	3 (100%)	2 (100%)

Table 4: Antibiogram of Enterobacteriaceae: shows sensitivity to different antibiotics

Antibiotic	**sensitive**	**%age**
Imipenem	228	97
Meropenem	214	91
Amikacin	214	91
Ceftazidime-clav acid	194	82.5
Ceftazidime	60	25.5
Cefrtiaxone	58	24.6
Gentamicin	196	83.4
Piperacillin-tazobactum	187	79.5
Ampicillin	74	31.4
Ciporfloxacine	97	41.2
Tigecycline	228	97

Diagram 2: Graph showing the %age of sensitivity to different antibiotics by Enterobacteriaceae



Discussion

In the present study 235 GNB were isolated from various clinical samples.

The distribution of GNB was shown in Table 1. *Escherichia coli* was most frequently isolated from 235 clinical specimen 107 (45.5%) followed by *Klebsiella spp* 73 (31%)

Similar findings were reported from Shankarankutty et al[1] *E.coli* 55.3% & *Klebsiella* spp 16.6%, Zaman et al[2]*E coli* 38.07% & *Klebsiella* spp15.91%, Mantravadi et al[6] reported 21.7% *E. coli* followed by 16.8% *Klebsiella* spp, Sahu et al[7] study showed *E coli* 58.5% & Klebsiella *spp* 41.5%, similar reports were noted by Vipin Kumar et al[8]*E coli* 58.4% &*Klebsiella* spp 22.2% in his study.

Other organisms like *Citrobacter*spp 28 (11.9%), *Proteus*spp 22 (9.3%), *Serratiamarcescens* 3 (1.2%), Enterobacter*arogens* 2 (0.8%) were reported in the present study.

Specimen wise distribution of GNB was shown in diagram 1 in the present study. Most of the GNB out of 235 samples were from urine sample 104 (44.2%) followed by pus sample 95 (40.4%). Similar findings were reported by Shankarankutty et al[1] 75.9% from urine sample & 55.3% from pus sample, Zaman et al[2] reported 43.7% from urine & 27.8% from pus , Sahu et al[7] noted 17.1% from urine followed by 11.1% from pus .

Organism wise distribution in various clinical samples was shown in table 2. *Escherichia coli* was most frequently isolated from urine sample 57 (53.2%) followed by pus 46 (42.8%), from sputum 2 (11.5%). *Klebsiell a*spp 28 (38.2%) from urine sample, 26 (35.6%) from pus, 11 (15%) from sputum, 6 (8.2%) from ET tube/catheter tip. Similar results were reported by Shankarankutty et al[1] 125 E.coli were isolated from urine sample followed by pus sample 21,Zaman et al[2] reported 73.1% E coli from urine sample & 39.2% Klebsiella spp from pus .

Citrobacterspp frequently isolated from pus 11 (39.2%), from urine 9 (32.1%). Proteus sppmore frequently isolated from urine sample 12 (54.5%), pus 9 (40.9%). Serratiamarcescens 3 isolates & Enterobacteraerogens 2 from pus sample.

Antibiotic sensitivity pattern of Enterobacteriaceae GNB was shown in table 3. In the present study *E coli* showed 98.30% sensitivity to carbapenem like Imipenem & 95.30% sensitivity to Meropenem, *Klebsiella* spp showed 94.5% sensitivity to Imipenem & 89% sensitivity to meropenem, similar findings were reported by Salma Nabi et al [9] as 98.89% sensitivity to Imipenem & 95.45% sensitivity to meropenem.

Serratiamarcescens & *Enerobacteraerogens* showed 100% sensitivity to carbapenems, piperacillin-tazobactum. *Citrobacter*spp & *Proteus*spp showed 92.8% & 90.9% sensitivity to carbapenems respectively, all Enterobacteriaceae showed sensitivity to tigecycline 90% - 100%,citrobacterspp showed 85.5% sensitivity to tigecycline in the present study.

Sensitivity to ciprofloxacine in E coli 57% and 19% in Klebsiella spp in the present study, Nabi et al [9] showed 56.7 % to *E coli*& 32.8% to *Klebiella* spp.

Overall percentage of sensitivity to different antibiotics by Enterobacteriaceae was shown in diagram 2. In the present study imipenem showed 95.7% and 89.9% sensitivity to meropenem, Tigecycline showed 95.7% sensitivity, Amikacin showed 89.9% sensitivity, Gentamicin showed 82.3% sensitivity, piperacillin-tazobactum showed 78.5% sensitivity, 3rd generation cephalosporins like ceftazidime 25.2%, cephotaxime 24.3% sensitivity. Nitrofurantoin specifically used for isolates from urine samples showed 95% senensitivity.

High amount of resistance was noted to ampicillin and 3rd generation cephalosporins in the present study, similar results were noted by Shankarankutty et al [1], also by Mohamaadmehr et al [10], Perisamy Hariharan et al[11].

Present study showed high sensitivity to amikacin and gentamicin, similar results were noted by Shankarankutty et al[1], senisitivity to ciprofloxacin was 42.2% in the present study, similar

results were noted by Perisamy Hariharan et al[11], Krithu Panta et al[12].

In the present study carbapenems, tigecyclinewer most active against multidrug resistant Enterobacteriaceae similar results were reported by Zaman et al[2], Krithu Panta et al[12]

Conclusion

Inappropriate and overuse of different antibiotics leads to antibiotic resistance in family Enterobacteriaceae. The present study and other published studies showed carbapenems are the drug of choice to treat multidrug resistant (MDR)-Enterobacteriaceae, but some strains of this group showed resistance to carbapenems also. It is very difficult to treat the infection showing resistance to carbapenems. The present study showed high resistance to ampicillin and 3rd generation cephalosporins. To overcome this every hospital should prepare antibiotic policy.

References

1. Jaya Sankarankutty, SaumyaKapu," Distribution and Antibiogram of Gram Negative Isolates from Various Clinical Samples at a Teaching Hospital, Tumkur", *Sch.J.APP. Med. Sci ,2014;2(3A): 927-931*.
2. Razina M Zaman, Magda M.Aly and Noof R Helmi,"Antimicrobial Susceptibility Pattern pf Gram-negative bacilli isolated from a Teaching Hospital in Jeddah, Saudi Arebia",Afr.J.Microbiol.Res.Vol.9(41),pp 2145-2158,14 October,2015.
3. Acaku Moses, Freddie Bwaga, Yap Boum and Joel Bazira,"Prevalence and Genotypic Charecterization of Exteneded Spectrm Beta – Lactamase produced by Gram Negative Bacilli at a Tertiary Care Hospital in Rural South Western Uganda", HHS Public Access.2014 December; 4(12):1541-1550,doi:10.9734/BMRJ/2014/9792.
4. Mita D Wadekar, K. Anuradha and D Venkatesha," Phenotypic Detection of ESBL and MBL in clinical isolates of Enterobacteriaceae",Int.J.Curr.Res.Aca.Rev.2013.
5. Clinical Laboratory Standards Institute (CLSI); Performance Standards for antimicrobial disc susceptibility testing, 26th edition, M100S,2016.
6. Hima Bindu Mantravadi, Mallikarjuna Reddy Chinthaparthi, ShravaniV," Aerobic isolates in pus and their antibiotic sensitivity pattern:a study conducted in a teaching hospital in Andhra Pradesh", Intrnational Journal of Medical Sciences and Public Health/2015/Vol 4/Issue 8:pp 1076-1079.
7. Sushil Kumar Sahu, A S Dalal, G Bansal,"Detection of extended spectrum β-lactamase in clinical isolates of E coli and Klebsiella species from Udaypur Rajasthan", Biomedical Research 2011;22(3):367-373.
8. Vipin K, Rohit K M, Avantika C, Pramila G ,"Incidence of β-lactamase producing gram negative clinical isolates and their antibiotic susceptibility pattern:A case study in Allahabad,"International Journal of Research in Pure and Applied Microbiology, 2011;1(3):36-39.
9. Salma Ghulam Nabi, Ghazal Zaffar, Shamim Mumtaz," Antimicrobial resistance pattern of gram negative bacilli Isolated from Clinical samples", Journal of Islamabad Medical and Dental College (JIMDC); 2014:3(1);25-28
10. Mohammadi-mehr M, Feizabadi M M," Antimicrobial Resistance Patern of gram negative bacilli isolated from patients at ICUs of Army Hospitals in Iran" Iranian Journal of Microbiology 2011;3(1):26-30.
11. Perisamy Hariharan, Thirunavukkarasu Bharani, Jonathan Sampath Franklyne, Prithwijit Biswas, Shaktiand Maneesh Paul-Satyaseeia,"Antibiotic susceptibility pattern of Enterobacteriaceae and non fermenter gram negative clinical isolates

of microbial resource orchid", J Nat Sci Biol Med V.6(1);Jan-Jun2015.

12. Kritu P Prakash G, Shiba K R,Reena K M, Ram N S, GaneshR," Antibiogram typing of gram negative isolates in different clinical samples of a tertiary hospital", Asian Journal of Pharmasuitical and clinical research,2013;6(1):153-156.