Original Article

Antibiotic sensitivity of Enterobacteriaceae at a tertiary care center in India

Abstract

Aims and Objectives: It has been observed that various microorganisms are acquiring resistance to most of the available potent antibiotics; hence, there is a need for every hospital to follow the use of antibiotics according to antibiotic sensitivity pattern in that particular hospital or geographical area. It has been reported that Enterobacteriaceae group of microorganisms are increasingly acquiring resistance to many antibiotics and this resistance varies geographically. As there is a short of recent data with respect to Indian hospital, this particular study was designed with the aim of establishing sensitivity pattern of Enterobacteriaceae group of microorganisms to various antibiotics. **Materials and Methods:** Data of antibiotic sensitivity from December 2010 to April 2011 of different Enterobacteriaceae was taken from the Department of Microbiology, Govt. Medical College, Surat. Sensitivity of different Enterobacteriaceae was shown as using descriptive statistics. **Results:** *E. coli* (55.6%) and *Klebsiella* (31.2%) were the most frequent bacteria isolated. Enterobacteriaceae were very less sensitive to amoxicillin + clavulanic acid (13.7%), chloramphenicol (7.6%), cefoperazone (14.4%), cefixime (15.7%), and cefuroxime (17.6). Sensitivity to aztreonam was 32.7%. Sensitivity to carbapenem group of drugs included in this study, i.e., *meropenem* was 69.8%. Highest sensitivity was shown for ceftazidime (74.1%). *E. coli* is more sensitive to meropenem as compared with *Klebsiella*. **Conclusion:** Sensitivity of Enterobacteriaceae group of microorganisms to known antibiotics is decreasing. Decreased sensitivity to carbapenem group of antibiotics is a matter of concern.

Key words:
Antibiotics, carbapenem, Enterobacteriaceae, resistance

Introduction

The fight between microorganism and mankind is going on since ages and both sides keep trying to discover new defense to combat each other. Microorganisms keep evolving new method of resistance to existed antibiotics and mankind's keep on finding new antibiotics. It is constantly observed that microorganisms are slowly getting supremacy in their method of evolving resistance and mankind is lagging behind in time in the discovery of new antibiotics.[1] Microorganisms are getting resistant to various antibiotics to whom they were sensitive before and therefore it is a matter of concern. An article published recently in Lancet Infectious disease revealed that Enterobacteriaceae samples from India are resistant to many antibiotics including the carbapenam which is considered as reserve drug for these microorganisms.[2] Some new reports suggest that Enterobacteriaceae, like *E. coli* and *Klebsiella*, are getting resistant to all known antibiotic including colistin and tigecycline.[3,4] So alarmingly, we are heading toward pre-antibiotic era, as in times to come there may be no availability of any antibiotic which can kill these microorganisms.[1] The various reasons for increasing antibiotic resistance in country like India could be irrational use of antibiotics, over the counter availability of higher antibiotics, poor sanitation, high prevalence of diarrhea, overcrowding and poor facility to conduct antibiotic sensitivity surveillance in hospitals.[1] Most of the hospitals including medical colleges have no proper implementation

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Summaiya Mulla, Jaykaran Charan[1], Tanvi Panvala

Departments of Microbiology and [1]Pharmacology, Govt. Medical College, Surat, Gujarat, India

Address for correspondence:
Dr. Jaykaran Charan,
Department of Pharmacology, Govt. Medical College, Surat, Gujarat, India.
E-mail: drjaykaran@yahoo.co.in

of antibiotic policy and irrational use of antibiotics in these hospitals is common.[5,6] After the study by Kumarasamy *et al*. got published in lancet infectious disease, debate again started in India regarding the problem of antibiotic resistance, especially in Enterobacteriaceae family of organisms which was highlighted in the study. The situation is more serious as newer antibiotics are not available in near future, particularly to this group of organisms.[2] This group of bacteria is responsible for several diseases like urinary tract infection, blood stream infections, hospital- and healthcare-associated pneumonias, intra-abdominal infections, gastroenteritis, etc. These drug-resistant Enterobacteriaceae are associated with high mortality and morbidity. In a study by Borer *et al*., it was observed that crude mortality and attributable mortality in the patients of carbapenemase-producing *Klebsiella pneumoniae* bacteremia was 71.9% and 50%, respectively.[7] In a similar study, it was observed that mortality among the cases with carbapenemase-resistant *K. pneumoniae* was significantly more as compared with control (40% *vs* 20%).[8] Due to the life-threatening infections caused by Enterobacteriaceae, their resistance to antibiotic is a serious issue of concern. There may be various ways by which Enterobacteriaceae acquire resistance, but production of extended-spectrum beta lactamase (ESBL) is more important. Carbapenem drugs are currently considered as the best treatment for these ESBL-producing Enterobacteriaceae. This group of drug is known to be stable to hydrolysis by beta lactamase and the nature of its chemical structure permits the drug for easy entrance through porin channel into bacteria. But, there are few studies which indicate emerging of resistance against carbapenems also.[2,3,9-11] This emerging trend of resistance in Enterobacteriaceae may lead to disastrous consequences with huge economic burden, as in years to come no antibiotics may remain effective. This may lead to profound mortality and morbidity in patients who would suffer from infection of these organisms. Steps need to be taken at many levels and one important step could be regular monitoring of these organisms for sensitivity to different antibiotics. As more and more bacteria are getting resistant to the antibiotics, periodic surveillance of sensitivity should be done in hospitals. It is observed that it is not routinely done in major hospitals including the medical colleges.[1] Antibiotic policy of the particular hospital should be based on antibiotic sensitivity profile of microorganisms. Keeping in mind that first step to solve a problem is to "acknowledge the problem" and so we tried to analyze antibiotic sensitivity pattern of Enterobacteriaceae group of bacteria in our hospital which is a tertiary level health center attached with medical college. The study is more relevant in the context of reporting of pan drug-resistant Enterobacteriaceae ("superbug") by Kumarasamy *et al*.'s study[2] and as sensitivity of Enterobacteriaceae to various antibiotics are not thoroughly studied in Indian context.

Materials and Methods

This study was conducted in Department of Microbiology, Govt. Medical College and New Civil Hospital, Surat, in the month of May, 2011. The sensitivity pattern of various microorganisms is routinely done on clinicians' request for hospital patients and the electronic record of the reports is routinely maintained in Microbiology Department. Samples are received from various clinical wards and outpatient departments with the request of antibiotic sensitivity tests against different antibiotics. These different samples (swab, pus, drain, urine, etc) are incubated overnight at 37°C temperature in different medias. After incubation, organisms are streaked over a Mueller Hinton agar plate. Antibiotic disc is placed in the middle of this Muller Hinton agar plate and it left for incubation at 37°C overnight. After overnight incubation, clear zone around the antibiotic disc is measured to know the sensitivity. All data related to antibiotic sensitivity are kept as excel sheet in microbiology department. We assessed these data retrospectively to know the trend in sensitivity pattern of different Enterobacteriaceae against many antibiotics. Microsoft excel 2008 was used for data cleaning. Double checking was done for any error in data filling in excel. Antibiotic sensitivity was done by disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guideline. All Enterobacteriaceae samples that underwent antibiotic sensitivity test between December 2010 and April 2011 were taken into consideration for data analysis.

Statistics

Descriptive statistics is used with the help of excel function. values are shown as frequency and percentage.

Results

Majority of samples were urine (30.9%), pus (23.7%), and swab (16.4%) [Table 1]. Enterobacteriaceae were isolated from 828 of the total samples; of these 828, number of samples with *E. coli* was 461 (55.6%), *Klebsiella* was 259

Table 1: Different samples collected for isolation of Enterobacteriaceae

Samples	Frequency (*n*=828)
Urine	256 (30.9)
Pus	197 (23.7)
Swab	136 (16.4)
Sputum	95 (11.4)
Drain	48 (5.7)
Tips	44 (5.3)
Blood culture	40 (4.8)
Fluid	8 (0.9)
Aspirate	2 (0.2)
Tissue	2 (0.2)

Values in parenthesis are percentages

(31.2%), Enterobacter was 66 (7.9%), Proteus was 28 (3.3%), *Salmonella typhi* was 7 (0.8%), Providentia was 4 (0.4%), and *Morganella* was 3 (0.3%) [Table 2].

Table 3 shows the antibiotic sensitivity of isolated Enterobacteriaceae. Enterobacteriaceae showed less sensitivity to amoxicillin + clavulanic acid (13.7%), chloramphenicol (7.6%), cefoperazone (14.4%), cefixime (15. 7%), and cefuroxime (17.6). Sensitivity to aztreonam was 32.7%. Sensitivity to carbapenem group drug included in this study, i.e., meropenem was 69.8%. Highest sensitivity was shown to ceftazidime (74.1%) [Table 3].

Table 3 shows antibiotic sensitivity of *E. coli* against various antibiotics. Very less sensitivity was shown to ceftriaxone (19.3%), ofloxacin (14.3%), norfloxacin (12.1%), amoxicillin + clavulanic acid (12.5%), cefotaxime (11.9%), chloramphenicol (6.7%), cefoperazone (12.3%), tetracycline (17.3%), cefixime (12.3%), and cefuroxime (12.7%). Sensitivity to meropenem was 80%. Highest sensitivity was shown for ceftazidime (84.5%) [Table 3].

Table 3 shows antibiotic sensitivity of *Klebsiella* against various antibiotics. Very less sensitivity was shown to amoxicillin + clavulanic acid (9.6%), cotrimoxazole (16.6%), cefotaxime (18.5%), chloramphenicol (7.3%), cefoperazone (15.4%), cefixime (15.8%), and cefuroxime (15.8%). Highest sensitivity of *Klebsiella* was shown to meropenem (55.5%) [Table 3].

Discussion

In this study, it was observed that *E. coli* and *Klebsiella* were the organisms isolated from majority of the samples and similar pattern of isolation were observed in other studies done with similar objectives.[12-15] These organisms are commonly associated with various blood stream infections including urinary tract infections; hence, their isolation is more in majority of the studies done with the similar objectives.

It was observed that penicillin group combinations like ampicillin + sulbactam and amoxicillin + clavulanic acid are not much effective against Enterobacteriaceae. Similar pattern was observed in other studies.[13-16] Organisms like *E. coli* and *Klebsiella* are intrinsically resistant to these antibiotics because of production of ESBL in them.[17] Resistance in Enterobacter may be because of production of AmpC beta lactamase.[17] ESBL producer organisms are usually resistant to many antibiotics. In a study done by Shahid *et al*. for prevalence of ESBL-producing bacteria in an Indian hospital, it was reported that 14.4% of *E. coli* and 24.6% of *Klebsiella* are ESBL producers.[18] Resistance to these group of antibiotics are associated with the overuse of these antibiotics in various infections, particularly urinary tract infection and easy availability of these antibiotics.[13,19,20]

In this study, it was observed that though sensitivity to first-generation and second-generation cephalosporins is

Table 2: Various Enterobacteriaceae isolated from samples

Organisms	Frequency (%)
E. coli	461 (55.6)
Klebsiella	259 (31.2)
Enterobacter	66 (7.9)
Proteus	28 (3.3)
Providentia	4 (0.4)
S. typhi	7 (0.8)
Morganella	3 (0.3)

Table 3: Antibiotic sensitivity of total Enterobacteriaceae, *E. coli*, and *Klebsiella*

Antibiotics	Sensitivity		
	Total Enterobacteriaceae (*n*=828)	*E. coli* (*n*=461)	*Klebsiella* (*n*=259)
Ampicillin + Sulbactam	310 (37.4)	173 (37.5)	84 (32.4)
Ceftriaxone	199 (24)	89 (19.3)	58 (22.3)
Ceftriaxone + Sulbactam	473 (57.1)	295 (63.9)	121 (46.7)
Ceftazidime	614 (74.1)	390 (84.5)	183 (70.6)
Levofloxacin	300 (36.2)	115 (24.9)	118 (45.5)
Ofloxacin	210 (25.3)	66 (14.3)	91 (35.1)
Netilmicin	253 (30.5)	89 (19.3)	103 (39.7)
Norfloxacin	187 (22.5)	56 (12.1)	85 (32.8)
Amoxicillin + Clavulanic acid	114 (13.7)	58 (12.5)	25 (9.6)
Cotrimoxazole	177 (21.3)	101 (21.9)	43 (16.6)
Cefotaxime	139 (16.7)	55 (11.9)	48 (18.5)
Chloramphenicol	63 (7.6)	31 (6.7)	19 (7.3)
Cefoperazone	120 (14.4)	57 (12.3)	40 (15.4)
Tetracycline	222 (26.8)	80 (17.3)	98 (37.8)
Ciprofloxacin	233 (28.1)	88 (19)	90 (34.7)
Gatifloxacin	342 (41.3)	214 (46.4)	82 (31.6)
Meropenem	578 (69.8)	369 (80)	144 (55.5)
Ticarcillin + Clavulanic acid	258 (31.1)	144 (31.2)	68 (26.2)
Cefixime	130 (15.7)	57 (12.3)	41 (15.8)
Cefepime + Tazobactam	257 (31)	131 (28.4)	74 (28.5)
Piperacillin + Tazobactam	512 (61.8)	307 (66.5)	129 (49.8)
Tobramycin	329 (39.7)	178 (38.6)	93 (35.9)
Cefoperazone + Sulbactam	568 (68.5)	357 (77.4)	141 (54.4)
Cefuroxime	146 (17.6)	59 (12.7)	41 (15.8)
Cefoperazone	249 (30)	121 (26.2)	69 (26.6)
Gentamicin	438 (52.8)	217 (47)	149 (57.5)
Ceftriaxone + Sulbactam	582 (70.2)	368 (79.8)	146 (56.3)
Cefepime	455 (54.9)	253 (54.8)	129 (49.8)
Aztreonam	271 (32.7)	136 (29.5)	76 (29.3)
Lomefloxacin	497 (60.0)	319 (69.1)	121 (46.7)

Values in parentheses are percentages

not rewarding, many Enterobacteriaceae including *E. coli* and *Klebsiella* were sensitive to ceftazidime. Sensitivity to ceftazidime as a whole is more as compared with even meropenem. Few studies show similar high sensitivity of Enterobacteriaceae to ceftazidime,[14,21] though it is observed in some studies that Enterobacteriaceae are increasingly getting more resistant to cephalosporins, including third-generation cephalosporins, particularly in *Klebsiella* group.[22-24] One of the reasons for this resistance may be production of ESBL by Enterobacteriaceae, particularly *E. coli* and *Klebsiella*.[25]

In this study, it was observed that sensitivity for different floxacillins (ciprofloxacillin, levofloxacillin, norfloxacillin, ofloxacin, and gatifloxacin) was less. One of the important reasons is overuse of these antibiotics for minor infections like urinary tract infections, etc. Similar observations were shown by other studies also.[14,26,27] As an exception in a similar study, most *E. coli* and *Klebsiella* were found to be sensitive to ciprofloxacillins.[17] It is observed in some other studies that resistance to ciprofloxacillin antibiotics are also escalating.[28] Resistance to gentamicin and netilmicin observed in this study is more as compared with study done by Gales *et al*. and Bartoloni *et al*.[29,30] but similar to study by Nwanje *et al*.[31]

One of the important findings of the study is decreased sensitivity to meropenem. Overall meropenem resistance was about 30%. *E. coli* was more sensitive than *Klebsiella*. The resistance to carbapenem group of drug, i.e., meropenem, in this study is much more as compared with other studies. In a study done by Wood *et al*., two surveillance databases were searched for imipenem or ertapenem resistance in Enterobacteriaceae. In Study for Monitoring Antimicrobial Resistance Trends (SMART) surveillance program, overall frequency of carbapenems was less than 2%. In ICU Surveillance Survey (ISS), resistance to carbapenem was less than 4%.[32] In these studies, it was observed that carbapenem resistance varies with geographical locations. In a similar study done in an Indian hospital, it was observed that most of the Enterobacteriaceae were sensitive to carbapenems. *E. coli* was 99% sensitive and *Klebsiella* was 100% sensitive to carbapenems. Carbapenems are considered as drug of choice for multidrug-resistant Enterobacteriaceae; hence, resistance toward these should be a matter of serious concern. Various reasons for resistance to Enterobacteriaceae are considered, like productions of ESBLs like AmpC, metallolectamases, etc., and losing of outer membrane.[33] These resistance genes are located on plasmid and can easily be moved from one organism to another through conjugation. These genes are associated with other drug resistance genes and move together.[2]

There is a need of doing similar studies in other hospitals to confirm and understand the resistance pattern of different Enterobacteriaceae, especially to carbapenems. There is also need to document geographic distribution of resistance. Antibiotic sensitivity analysis is to be made a routine activity in any hospital and prescription of antibiotics should be guided by the pattern of antibiotic sensitivity. There is a need of initiating some policy changes to prevent such resistance against highly effective drugs like meropenems and other carbapenems. Situation is more bothersome as the discovery of new antibiotics to tackle Enterobacteriaceae group of microorganisms are at a slower pace than necessity. Only few antibiotics like colistin and tigecycline are known to be effective against these carbapenam-resistant Enterobacteriaceae, but these drugs have low safety profile and associated with attributable mortality in patients treated by them. And, few reports suggest resistant to these colistin and tigecycline too in Enterobacteriaceae.[34] So at present, there is no safe antibiotic available for the treatment of carbapenem-resistant Enterobacteriaceae and this is really of concern.

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