Frequency of isolation and susceptibility pattern of E. coli from in and outpatients over a period of five years at The Indus Hospital Network, Karachi Pakistan

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Abstract

Objective: To determine the frequency of isolation and susceptibility pattern of Escherichia coli at a urban health centre.

Method: The retrospective study was conducted at the Indus Hospital Network, Karachi, and comprised Enterobacteriaceae isolates obtained from urine, blood and routine culture from patients presenting between 2013 and 2017. The samples were evaluated for resistance patterns against a range of antibiotics and frequency of isolation was determined. Data was analysed using SPSS version 21.0

Results: Of the 10,667 isolates analysed, 6380(60%) were Escherichia coli. Outpatient and inpatient isolates constituted 4184(65.6%) and 2196(34.4%) of the total E. coli isolates respectively. Of the 1446(22.66%) isolates obtained from urine, 1007(59.64%) had E. coli which was the highest isolation rate among all types of samples. There was a significant difference in resistance rates between inpatient and outpatient isolates for first-line and second-line injectable and oral antibiotics ($p<0.05$).

Conclusion: Escherichia coli isolates showed high resistance towards co-amoxiclav, ampicillin, ceftriaxone and ciprofloxacin, leaving little empirical options for treating outpatients.

Keywords: Susceptibility pattern, E. coli, Pakistan.

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Introduction

Enterobacteriaceae is a large family of gram-negative bacteria that includes Escherichia (E.) coli, a dominant member of the normal gut flora of vertebrates. E. coli also accounts for 17.3% of clinical infections that require hospitalisation and is the second most common source of infection next to staphylococcus (S.) aureus in outpatient infections,[1] showing that it can form symbiotic relationships at one location and assume a pathogenic role at another site of the body. Its ubiquitous presence in the body and environment exposes it to elevated use of antibiotics that has resulted in an exponential growth of antimicrobial resistance, causing inpatient and outpatient infections.[2-4] Commensal E. coli can spread and take up bacterial genetic elements, including plasmids that encode antibiotic resistance genes, thus propagating the growth and spread of antibiotic resistance while selecting for the development of bacteria that are resistant to the antibiotic being consumed.[5,6] Therefore, the rise of antimicrobial resistance is a major concern for clinicians worldwide.[4]

Previous studies have shown a higher prevalence of antimicrobial resistance in inpatients than in the community/outpatients.[7] A high selective pressure for broad spectrum antibiotics towards inpatient isolates has resulted in a greater increase in antibiotic resistance than for outpatients. The approach being used to curb antimicrobial resistance mainly pertains to the implementation of antibiotic stewardship.[8]

The emergence of drug-resistant pathogens complicates the treatment of infections. Approximately 95% of cases with severe symptoms are treated without bacteriological investigation and the frequency of isolation and susceptibility patterns of E. coli vary geographically as well as across populations and regions.[9] It supports the fact that when clinicians are aware of resistance and prevalence, more prudent antibiotic prescription can take place for empirical treatment.[10]

The current study was planned to determine the magnitude of antimicrobial resistance at an urban healthcare centre, and to compare inpatient and outpatient data to identify resistance patterns of E. coli.

Materials and Methods

The retrospective study was conducted at the Indus Hospital Network, Karachi, and comprised Enterobacteriaceae isolates obtained from urine, blood and routine culture from inpatients and outpatients presenting between 2013 and 2017. The samples related to pus, tissue, sputum, trachea etc. After obtaining exemption from the institutional ethics review board, data was extracted using the Hospital Management Informatics System. Duplicate isolates within a month's timeframe were excluded. Blood

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and routine samples were inoculated on chocolate, blood agar and MacConkey agar (Oxoid, UK), while urine specimens were plated on cysteine lactose electrolyte deficient (CLED) agar (Oxoid, United Kingdom [UK]). They were subsequently incubated aerobically at 37^{O}C for 24-48 hours. After incubation, the cultures were examined for significant growth. In urine samples, a significant bacterium was considered if culture yield was ≥10^5 CFU/mL. Identification of the microorganisms was done through gram staining and biochemical tests, including catalase test, citrate utilisation, oxidase, indole production, motility, urease and triple sugar iron test (Oxoid, UK).

Once the genus of the organism was identified, antibiotic susceptibility tests were performed using the Kirby and Bauer disc diffusion method and the results were determined according to the Clinical and Laboratory Standards Institute (CLSI) antibiotic guideline.[11]

Antimicrobial susceptibility was performed on Mueller-Hinton agar. The tests and the disc content used included oral antibiotics amoxicillin-clavulanate 20/10µg, trimethoprim-sulfamethoxazole 1.25ug/23.75ug, nalidixic acid 30ug, ciprofloxacin 10µg, fosfomycin, nitrofurantoin, and norfloxacin 10µg. First-line injectables included gentamycin 10µg and ampicillin 10µg. Second-line injectables included amikacin 30µg, ceftazidime 30µg, ceftriaxone 30µg, cefotaxime 30µg, meropenem 30µg, imipenem 10µg, tetracycline 30µg, piperacillin-tazobactum 100/10µg and cefoperazone-sulbactum 30/10µg. All plates for antimicrobial susceptibility testing were incubated at 35-37^{O}C in 5% carbon dioxide (CO_2) for 24 hours. E. coli (ATCC25922) was used as the control strain. Zone diameters were measured and interpreted as per the CLSI 2017 guidelines.[11]

Data was analyzed using SPSS version 21.0. Chi-square test was used for statistical comparisons between groups and years. P<0.05 was considered statistically significant.

Results

Of the 10,667 isolates analysed, 6380(60%) were E. coli. Outpatient and inpatient isolates constituted 4184 (65.6%) and 2196(34.4%) of the total E. coli isolates respectively. Of the 1446 (22.66%) isolates obtained from urine, 1007 (59.64%) had E. coli which was the highest isolation rate among all types of samples Table 1).There was a significant difference in resistance rates between inpatient and outpatient isolates for first-line and second-line injectable drugs as well as oral antibiotics (p<0.05) Cefoperazone-sulbactam showed high resistance in inpatients compared to outpatients (p<0.001). The antimicrobial resistance was significantly higher in inpatient isolates for ampicillin, ceftriaxone and gentamicin compared to the outpatient isolates (p<0.001). Nitrofurantoin, fosfomycin, amikacin and tetracycline showed no significant difference (p>0.05). Amoxicillin-clavulanate, ciprofloxacin, nalidixic acid and trimethoprim sulpha-methoxazsole were significantly different in terms of resistance (p<0.001) (Table 2).

Table-1: Total and individual frequency of isolation of Escherichia (E.) coli in different samples.

	EB	E. coli n (%)
Outpatient		
Blood Culture	294	42 (14.29)
Routine	772	306 (39.64)
Urine	5800	3836 (66.14)
Total	6866	4184 (65.6%)
Inpatient		
Blood Culture	440	150 (34.10)
Routine	1915	1039 (54.30)
Urine	1446	1007 (69.64)
Total	3801	2196 (34.4%)
Total Isolation	10667	6380 (59.81063)

EB: Enterobacteriaceae

Table-2: Resistance to specific antimicrobials in Escherichia (E.) coli isolates from inpatients and outpatients.

	Antibiotic Susceptibility Pattern in E. coli from 2013-2017 (%)		
	Inpatient	Outpatient	p value
Oral Antibiotics			
Amoxicillin-clavulanate	86	74	<0.01
Ciprofloxacin	72	66	<0.01
Nalidixic Acid	91	79	<0.01
Trimethoprim-sulfamethaxazole	75	67	<0.01
Fosfomycin	7	6	0.409
Nitrofurantoin	12	11	0.127
Norfloxacin	76	69	<0.01
First Line Injectable			
Ampicillin	95	90	<0.01
Ceftriaxone	84	76	<0.01
Gentamicin	40	32	<0.01
Second Line Injectable			
Imipenem	6	2	<0.01
Meropenem	6	2	<0.01
Piperacillin-tazobact	31	15	<0.01
Norfloxacin	76	69	<0.01
Cefoperazone-sulbactam	49	40	<0.01
Colistin	1	1	0.558
Amikacin	5	4	0.016

Discussion

Antimicrobial resistance in E. coli has increased worldwide and its susceptibility patterns vary significantly across

geographic settings, populations and environments.[12]

To the best of our knowledge, the current study is the first tp explore E. coli's susceptibility pattern between inpatient and outpatient isolates in Karachi. The E. coli isolation rate was 60% and it was most commonly isolated from urine samples for both types of patients. Consistent with other reports, we found that E. coli showed relatively higher resistance rates in inpatient than outpatient isolates for all antibiotics.[13-18] The differential increase in the rate of resistance between inpatient and outpatient isolates urges auditing the susceptibility pattern that allows for an empirical selection of antibiotics.

Research studies have demonstrated an association between antimicrobial use and resistance.[15,16] The proportion of patients receiving antimicrobials is much higher in hospitals, and, hence, explains the difference between inpatient and outpatient isolates.

Low resistance rates towards amikacin were observed from E. coli which was consistent with a study in India where only 5% prevailence was reported. E. coli isolates had very high sensitivity towards gentamicin in northeastern Ethiopia compared to our study which showed 40% resistance, reinforcing the difference in susceptibility patterns in regions and also deterring the use of this antibiotic for E. coli in Karachi.[9] Our results elucidate that the resistance trend for gentamicin has decreased over five years, suggesting that it was used frequently in this region of Karachi.

Penicillin, as a group of antibiotics, is cheap and available over the counter in Pakistan. Self-medication is a common practice for ailments like ear, eye, wound infections as well as fever.[19] Consequently, there is increasing resistance to these antibiotics as is evident from our study, where the resistance to ampicillin was 95% and 90%), amoxicillin-clavulanate 86% and 74% in inpatients and outpatients respectively. Such high resistance rates render these antibiotics inapt for empirical therapy for inpatients as well as for community-acquired infections caused by E. coli. These antibiotics have shown decreased susceptibility towards E. coli within the last 10 years in Pakistan.[20,21] Our findings related to inpatient and outpatient isolates having high resistance to amoxicillin-clavulanate is similar to other reports.[9]

Our study indicates a high resistance rate for injectable cephalosporin (ceftriaxone) for inpatient (84%) and outpatient (76%) isolates. This may be due to the prescription of injectable antibiotics for illness like acute diarrhoea and fever without true knowledge of the causative agent.[22] In recent years, use of fluoroquinolones (ciprofloxacin and norfloxacin) has increased in many countries, and the resistance of bacterial isolates to fluoroquinolones has been observed.[23,24] Nitrofurantoin demonstrated better activity against E. coli isolates with 11-12% resistance between inpatinets and outpatients. Regular monitoring and evaluation of antibiotic susceptibility pattern is recommended to establish a preventive and therapeutic guideline for healthcare professionals.

Conclusion

E. coli showed high rates of antimicrobial resistance to ampicillin, co-amoxiclav, ceftriaxone, ciprofloxacin, sulphamethoxazole and trimethoprim. Therefore, nitrofurantoin, fosfomycin, carbapenems and amikacin should be considered for empirical treatment of E. coli in the study area with cautious consideration of the site of infection and associated complications.

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