Comparative analysis of Amikacin, Ceftriaxone, Ciprofloxacin and Imipenem against Escherichia coli and Salmonella typhi: A prospective experimental study

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ABSTRACT

Objective: To analyze and compare activity of four different antibiotics namely Amikacin, Ceftriaxone, Ciprofloxacin and Imipenem against Escherichia coli and Salmonella typhi.
Study Design: A prospective experimental study.
Place and Duration: At Pharmaceutical Microbiology Lab, Department of Pharmaceutics, Hamdard University, Karachi, Pakistan from December 10, 2017 to May 18, 2018.
Methodology: Kirby-Bauer's Disk Diffusion method was employed for antibiotic testing and One-way ANOVA determined significant differences of zone of inhibitions (at $p<0.05$).
Results: High resistance was shown towards Ceftriaxone both by E.coli and S.typhi strains as 67% and 50.94% respectively. 91.75% susceptibility to Imipenem was noted for E.coli and that of 75.47% was shown by S.typhi.
Conclusion: Higher susceptibility patterns were noted towards Imipenem and maximum resistivity was highlighted against Ceftriaxone by both E.coli and S.typhi amongst all the four tested antibiotics. Ciprofloxacin and Amikacin exhibited moderate activity against both E.coli and S.typhi.
Keywords: Antibiotics, Amikacin, Ceftriaxone, Ciprofloxacin, Imipenem, E.coli, S.typhi, Sensitivity, Resistance, Disk diffusion, Zone of Inhibition.

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INTRODUCTION

The antibiotics resistance has become a serious global concern, threatening human health. Within the past few years, Enterobacteriaceae has acquired resistance all over the world[1].

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South-Asia is known to be the hub for multidrug-resistant (MDR) bacteria and within Pakistan, multi-drug resistant Enterobacteriacea (resistant to Chloramphenicol, Ampicillin and Co-trimoxazole) is increasing at an alarming rate[2]. Managing and preventing of these resistant enteric strains in developing countries become even harder as they also prolong the illness and increase complications rate[3].

It is expected that resistance enteric strains including E.coli and S.typhi will continue to grow in Karachi, Pakistan. This is because, for over the past decade, there has been no significant progress in reduction of infectious diarrhea[4]. Morever, when compared with other Asian countries, India and Pakistan possess the highest incidence rates of acute gastroenteritis and enteric fever as 214.2 and 451.7 cases, on each population of 100,000, respectively[5]. It may be hard to manage such outbursts of MDR Salmonella (S.) Typhi, typically in developing countries where resources are limited[6].

Other than the first-line antibiotics, the resistance of S.typhi is also reported to be increasing towards Fluoroquinolones and Cephalosporins all over the world and Karachi is also suffering from these resistant strains[7]. The strains of E.coli have also been found to be resistant to fluoroquinolone (Ciprofloxacin) in Karachi[8]. Resistant strains can be treated with third-generation Cephalosporins as Cephalosporins are proven to be safely used against E.coli[9]. However, resistance to third-

generation Cephalosporins is also evolving[10]. In a single-center prospective analysis conducted in Karachi, it was found that strains of E.coli showed sensitivity towards Amikacin and Imipenem but were resistant to Ceftriaxone and Ciprofloxacin[11].

The crisis of antibiotic resistance particularly in a developing country like Pakistan, has become prevalent due to misuse and over-use of antibiotics[12]. In a cross-sectional study conducted in Karachi, it was reported that the leading factor for growing antibiotics resistance in this city is due to lack of knowledge among local population regarding antibiotics use and irrational antibiotics prescription by physicians[13]. Combined with the threat of poor sanitation, unsafe water supply, irrational antibiotic use and growing antibiotics resistance, the treatment of common prevailing enteric infections in our community is an imperative future concern. The need is to stop emerging resistance by doing testing of resistance and sensitivity patterns at regular intervals for the guidance of clinicians at the local level[14].

Therefore, main objective of this study is to analyze and compare activity of four different antibiotics namely Amikacin, Ceftriaxone, Ciprofloxacin and Imipenem against Escherichia coli and Salmonella typhi.

METHODOLOGY

This prospective experimental study was conducted at Pharmaceutical Microbiology Lab, Department of Pharmaceutics, Hamdard University, Karachi. Sample size of study is calculated by precision analysis technique[15]. A total of 150 clinical isolates (E.coli and S.typhi) had been collected from different pathologic laboratories of Karachi over a period of December 10, 2017 to April 05, 2018. E.coli were obtained from the fecal samples, responsible for causing infectious diarrhea or gastroenteritis and S.typhi had been acquired from the blood specimens responsible to cause Enteric or Typhoid fever. Clinical isolates obtained from any other specimen other than stool or bloods were excluded.

These isolates were obtained from both genders and were inclusive of all ages. After isolates collection, performance of antibiotics testing was executed. The examination of morphology within the medium MacConkey agar helped us in identifying the bacterial strains. The antibiotics tested against clinical isolates of E.coli and S.typhi were Amikacin, 30mcg, Oxoid (CT0107B), Ceftriaxone 30mcg, Oxoid (CT0417B), Ciprofloxacin 5mcg, Oxoid (CT0425B) and Imipenem 10mcg, Oxoid (CT0455B). Kirby-Bauer's disk diffusion method was employed for testing of the antibiotics sensitivity and resistance, following of the guidelines provided by Clinical and Laboratory Standard Institute[16]. Already prepared and preserved slants of Tryptone Soy Agar at 40^0C were used for the inoculation of the isolates. In order to get the turbidity required as that of the prepared 0.5 McFarland standard, tubes were incubated at a temperature of 35-37^0C for a few hours until the pre-requisite turbidity was attained[17]. Within not more than 15 minutes of the antibiotics disc placement, the plates were incubated at a temperature of 35-37°C for 24 hours[18]. The zone of inhibition was measured by Vernier caliper, and interpretation of the antibiotic sensitivity was performed following the guidelines provided by CLSI[19].

Data Analysis: The data was examined by IBM (International Business Machines Corporation) SPSS (Statistical Package for the Social Sciences) 22 software. One-way ANOVA (Analysis of Variance) was employed in order to examine the significant difference in zone of inhibition among all of the four antibiotics. The data was also examined for its homogeneity and normality. At a value of $p<0.05$, a difference was regarded as a significant one.

RESULTS

A total of 150 isolates were tested against four antibiotics, out of which 97 were E.coli and 53 were S.typhi. As per results interpreted on the basis of CLSI guidelines, varying sensitivity and resistance patterns of both E.coli and S.typhi against all four tested antibiotics were noted (Figure-1).

In Figure-1, Maximum susceptibility of E.coli towards Imipenem is pre-dominant with 91.75% (n=89) susceptibility, followed by a significant sensitivity of 60.82% (n=59) towards Amikacin. A higher resistance of 67.01% (n=65) exhibited by E.coli against Ceftriaxone is also noteworthy and is dominating among all the displayed resistance patterns in this figure I. For S.typhi, equal patterns of 75% (n=40) susceptibility towards Imipenem and Amikacin is highlighted, whereas its maximum resistance against Ceftriaxone is also dominating with a resistivity degree of 50.94% (n=27). Further resistance and susceptibility patterns of both E.coli and S.typhi are highlighted in Figure II and III.

From Figure-2, moderate resistance and susceptibility patterns of E.coli towards Amikacin and Ciprofloxacin can be clearly seen. Minimal resistance of 3.09% (n=3) to Imipenem among all four antibiotics is noteworthy. Ceftriaxone is dominating in terms of its resistance and E.coli exhibited only 14.43% (n=14) susceptibility to it. Towards Amikacin, a comparatively lesser degree of resistance was exhibited by E.coli with 23.71% (n=23) resistivity. However, Ciprofloxacin had not shown any remarkable activity as it remained average, with 26.80% resistance (n=26) and 43.30% (n=42) susceptibility, towards it.

Figure-3 highlights an equal susceptibility pattern of S.typhi towards Imipenem and Amikacin which is 75.47% (n=40). Moreover, 0% (n=0) resistance to Imipenem is also worth notifying, signifying its credibility to be a suitable effective antibiotic option against S.typhi. Ceftriaxone, however, is indicated to show the higher degree of resistivity among all four antibiotics and only 33.96% (n=18) susceptibility was exhibited towards it. Ciprofloxacin, on the other hand, did not come up with any considerable resistivity or susceptibility pattern and thus its activity remained insignificant with 20.75% (n=11) susceptibility, and 39.62% (n=21) resistance.

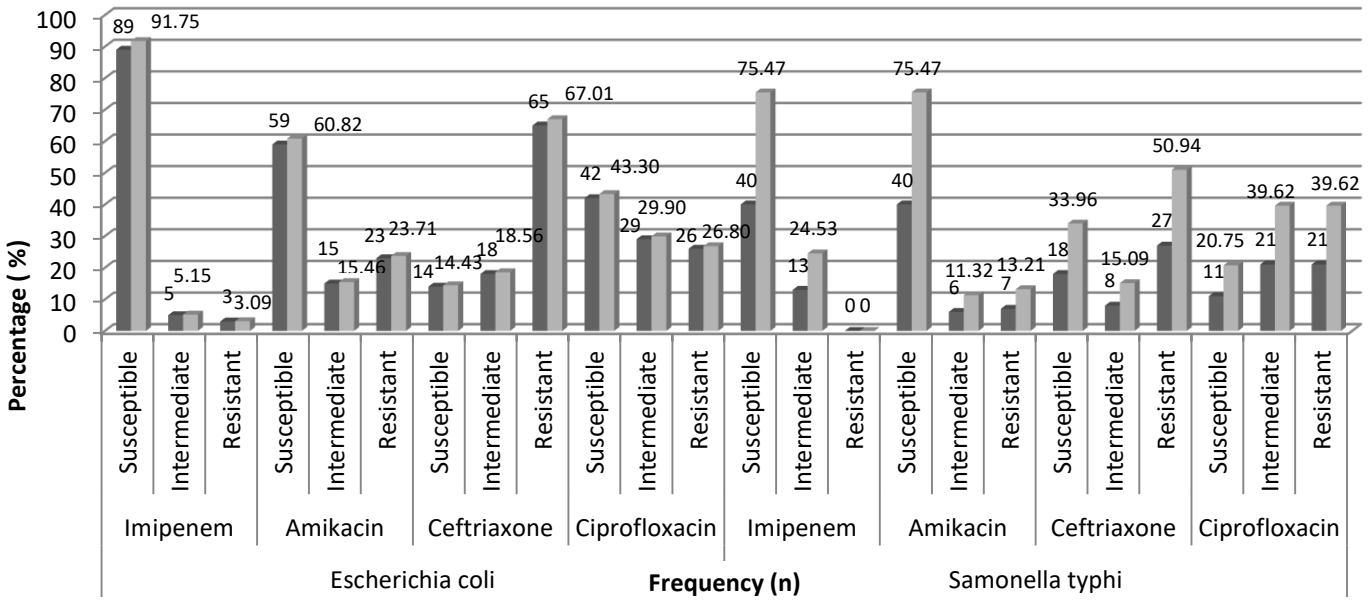
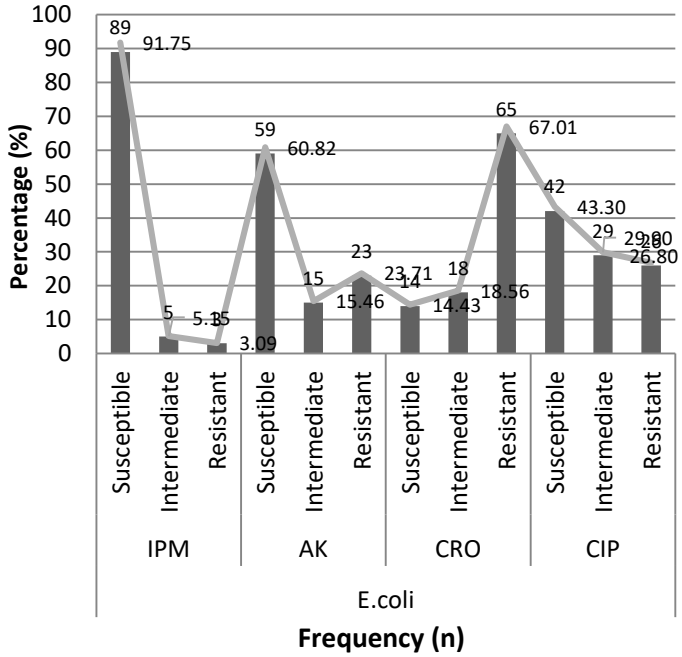


Figure-1: Comparative Activity of All Four Antibiotics against Isolates (N=150)

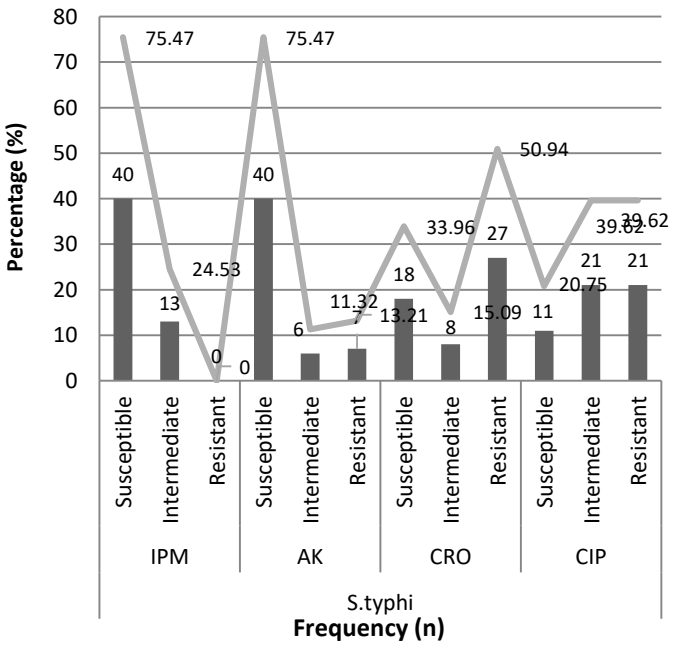


IMP: Imipenem, AK: Amikacin, CRO: Ceftriaxone, CIP: Ciprofloxacin

Figure-2: Resistance and Sensitivity Patterns of E.coli Against Four Antibiotics (n=97)

Significant differences among the zone of inhibitions of all four antibiotics against E.coli and S.typhi have been revealed by applying of ANOVA (Table-I). Level of significance is <0.05%.

The Least Significant Difference Test (LSD) is the most common test applied to determine significant differences among the means. It is the most simple and widely used tool, belonging to the post hoc test, proposed by Fisher in 1935. It depends upon the minimum difference between the two means that is considered to be significant at a particular significance level[20]. Here, the level of significance is kept at <0.05. Retrieved from the table-I analysis, it is revealed that zone of inhibition against both E.coli and S.typhi by Imipenem is significantly (p=0.0001) better than Amikacin, Ceftriaxone and Ciprofloxacin. This means that Imipenem showed better zone of inhibitions among all four antibiotics for both E coli and S typhi. Amikacin and Ciprofloxacin also revealed considerably better zone of inhibitions as compared to Ceftriaxone. Thus, Ceftriaxone was identified as the most resistant one, whereas Imipenem was the most susceptible antibiotic among all four tested antibiotics.



IMP: Imipenem, AK: Amikacin, CRO: Ceftriaxone, CIP: Ciprofloxacin

Figure-3: Resistance and Sensitivity Patterns of S.typhi against Four Antibiotics (n=53)

Table-I: Comparison of Mean differences in Zone of inhibition of E.coli (n=97) and S.typhi (n=53) Against Four Antibiotics (N=150)

Antibiotic	Comparator	Mean difference in zone of inhibition	Significance at < 0.05
	E.coli		
Imipenem	Amikacin	13.35	p=0.0001
	Ceftriaxone	15.83	p=0.0001
	Ciprofloxacin	11.13	p=0.0001
Amikacin	Ceftriaxone	2.48	p=0.0080
	Ciprofloxacin	-2.21	p=0.0170
Ceftriaxone	Ciprofloxacin	-4.70	p=0.0001
	S.typhi		
Imipenem	Amikacin	8.84	p=0.0001
	Ceftriaxone	9.79	p=0.0001
	Ciprofloxacin	4.01	p=0.0020
Amikacin	Ceftriaxone	0.943	p=0.4690
	Ciprofloxacin	-4.83	p=0.0001
Ceftriaxone	Ciprofloxacin	-5.77	p=0.0001

DISCUSSION

The mean zone of inhibitions of the four antibiotics against both E.coli and S.typhi were compared by employing ANOVA (Analysis of Variance). ANOVA determines if there is a significant difference exist between means of different groups that may be two or more than two in number[21]. Moreover, post-hoc test was applied to identify how the four groups of antibiotics are differing from each other by comparing different pairs of antibiotic groups[22]. Analysis reveals that zone of inhibition made by Imipenem against both E.coli and S.typhi is significantly better (p=0.0001) than Amikacin, Ceftriaxone and Ciprofloxacin. The highest mean difference in zone of inhibition was observed between Imipenem and Ceftriaxone as 15.83mm and 9.79mm for E.coli and S.typhi respectively. This is indicative of the difference in the activities of both these antibiotics, the former being most sensitive and the latter most resistive.

The maximum resistance to Ceftriaxone exhibited by both E.coli and S.typhi strains is the most dominating aspect of our study results. Based upon results generated through CLSI guidelines, 67% of E.coli strains were found Ceftriaxone resistant. Interestingly in another study conducted in Karachi, 67% resistant to ceftriaxone was shown by E.coli,[23] showing consistency with our results. Similarly, S.typhi revealed maximum resistance 50.94% to Ceftriaxone among all the four antibiotics. The first case of Ceftriaxone-resistant typhoid fever were identified in Hyderabad, Pakistan in November 2016 in which more than 50% resistance was shown towards Ceftriaxone by S.typhi strains[24], which is in agreement with our results. Furthermore, three hundred and thirty-nine isolates with the similar XDR (Extreme Drug Resistant) pattern were acquired from the Sindh region, Pakistan[25].

Our study results combined with the other studies in this regard, signify the emerging threat of antibiotic resistance in S.typhi found in Karachi.

Resistance to third-generationcephalosporins leaves clinicians with limited drug choices for treating gram-negative infections except Carbapenem[26]. Our study revealed Imipenem to be highly sensitive against both the E.coli and S.typhi. E.coli showed 91.75% sensitivity to it, and that of S.typhi was shown as 75.47%. Carbapenems are considered as the "last-line agents" in case of patients suffering from multi-drug resistant bacteria[27]. This shows Imipenem credibility to keep it as a last treatment option. In a study conducted at a pediatric unit of a tertiary care hospital of Karachi, E. coli was the most common pathogen isolated in 60% stool samples out of the total 152 isolates. The most sensitive antibiotics against E.coli, reported in that study was Imipenem with 97% susceptibility[24]. This is in agreement with our study as, out of the 150 samples collected, 97 belonged to E.coli, which reveals a high prevealnce of infectious diarrhea within our locality. Morever, most sensitive antibiotic for E. coli was highlighted as Imipenem with 91.75% sensitivity.

After Imipenem, the highest susceptibility was shown towards Amikacin by E.coli as 60.82%. Salmonella typhi displayed similar sensitivity pattern for Amikacin which was 75.47%. This is in consistency with results of another study conducted in Karachi in which 97.8% susceptibility was revealed by E.coli towards Amikacin[24].

Both E.coli and S.typhi revealed a comparatively lower susceptibility rate 43.30% and 20.75% to Ciprofloaxcin among all four antibiotics. In another study, conducted in this regard, all the E.coli isolates exhibited absolute (100%) sensitivity to ciprofloxacin[28], which is in contrast to our study. In another study conducted in Karachi, ciprofloxacin resistance was 27.02% and 16.66%, to Escherichia coli and Salmonella typhi respectively. Variation in these results highlights how rapidly ciprofloxacin resistance is rising in our region. High resistance development against ciprofloxacin might be due to its irrational and inappropriate use[8].

It has been acquired from this study that a rapidly growing resistance to Ceftriaxone and Ciprofloxacin among the local population of Karachi is alarming. It raises questions on the indiscriminate use of antibiotics particularly on the prescription patterns of third generation Cephalosporins.

CONCLUSION

Higher susceptibility patterns were noted towards Imipenem and maximum resistivity was highlighted against Ceftriaxone by both E.coli and S.typhi amongst all the four tested antibiotics. Ciprofloxacin and Amikacin exhibited moderate activity against both E coli and S typhi.

AUTHOR'S CONTRIBUTION

Najeeb D: Conceived idea, Manuscript writing
Khaliq SA: Study supervisor, Designed research methodology, Final drafting of manuscript.
Fatima B: Co-supervisor, Literature review, Statistical analysis

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