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Research Article

Comparative Study on Resistance Pattern of Clinical Isolates of Severe Acute Pancreatitis Against Moxifloxacin and Ciprofloxacin: An *In Vitro* Study

Samiullah Burki[1], Muhammad Iqbal Nasiri[*2], Sohail Anwer[2], Rizwan Ahmad[3], Kamran Ahmed[2]

[1]*Department of Pharmacology, Faculty of Pharmacy, Hamdard University, Karachi –76400, Pakistan*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Karachi –76400, Pakistan*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Hamdard University, Karachi –76400, Pakistan*
[*]***Correspondence:*** iqbalnasiri@hotmail.com (Tel.: +923343377642)

Abstract

The objective of this research work was to study the potential sensitivity and resistance pattern of moxifloxacin and ciprofloxacin on the isolated pathogens of severe acute pancreatitis (SAP) in a cohort population. For this purpose, a total of 140 isolates were collected from different Public hospitals of Karachi. In vitro activity was determined by agar dilution method by using moxifloxacin and ciprofloxacin. The concentrations of these antibiotic used were 0.5 mg/L, 1 mg/L, 2 mg/L, 4 mg/L, 8 mg/L, 16 mg/L. In this study, the most common pathogens associated with SAP were *Escherichia coli* 39%, *Pseudomonas aeruginosa* 32%, *Staphylococcus aureus* 20%, and *Klebsiella* spp. 10%. The concentrations of both drugs i.e.; Moxifloxacin and Ciprofloxacin were in range of 0.5mg/L to 16mg/L. Escherichia coli (11% against moxifloxacin and 17% against ciprofloxacin), *Staphylococcus aureus* (18% against moxifloxacin and 39% against ciprofloxacin), *Klebsiella* species (7% against Moxifloxacin and 14% against ciprofloxacin), and *Pseudomonas aeruginosa* (48% against moxifloxacin and 50% against ciprofloxacin), at concentration of ≤ 16mg/L were found resistant against these two antibiotics which showed that moxifloxacin is more potent and effective as compare to ciprofloxacin against these isolates. However, *Pseudomonas aeruginosa* was the only pathogen which showed marked resistance against the both antibiotics. It is concluded that, the clinical isolates collected were susceptible against both the antibiotics but the resistance pattern is increasing in our population which is a threat in the future. Therefore, it is recommended that the physicians may prescribe these antibiotics unless no other substitute is available in clinical practice. Thus, the moxifloxacin may potentially be effective chemotherapy in the management of severe acute pancreatitis and other complications of pancreas.

Keywords: Clinical isolates, severe acute pancreatitis, moxifloxacin, ciprofloxacin

Introduction

Severe acute pancreatitis (SAP) is a life threatening condition. Particularly, in third world countries like Pakistan where poor diagnosis and inappropriate chemotherapy leads to microbial resistance and develop a chance of relapse of SAP. This disorder is characterized by inflammation. Infection is common condition in severe acute pancreatitis which increase mortality rate threefold [1]. Management of severe acute pancreatitis with antimicrobials is still under discussion. It is because severe acute pancreatitis is not only acute inflammatory process but also involve other regional tissue which leads to necrosis of pancreas followed by pancreatic abscess. This complicated case of severe acute pancreatitis increases the chance of mortality [2]. Most of the cases showed that gram negative aerobic pathogens are involved in severe acute pancreatitis, which usually arise from normal flora thus so called endogenous infection [1]. Different clinical studies showed that the translocation of these gram negative microorganisms in pancreas occur through hematogenous circulation [3-4] and also commonly through Colon [5, 6], it can also enter to Circulatory System through Lymphatic [7] through ascites to pancreas [4, 7]. It can also be translocate from small intestine through bile or to the main pancreatic duct [8] and it can also penetrate pancreas from duodenal chyme reflux [9]. These predominant gram negative microorganisms include *Escherichia coli* (*E.coli*), *Pseudomonas*, *Klebsiella* spp., *Proteus* spp., *Streptococcus faecalis*, *Enterobacter* spp [10]. Similar, key microorganisms including *E.coli*, *Pseudomonas aeruginosa, Klebsiella* speices and gram positive *Staph. aureus*, were reported by [11] during various studies of SAP. Different antibiotics are used for the management of severe acute pancreatitis. Quinolones are extensively used in acute pancreatitis and other intra-abdominal infection because of their wide spectrum. In Pakistan, patients with severe acute pancreatitis are shifted to newer quinolones, because lower respiratory tract infection is very common, usually treated by doctor in Pakistan easily. In current study, clinical isolates of severe acute pancreatitis were collected and these isolates were evaluated for their resistance pattern against Moxifloxacin and Ciprofloxacin. Moxifloxacin and Ciprofloxacin belong to 4th and 2nd generation fluoroquinolone, respectively. Moxifloxacin is a broad spectrum antibiotic and have efficient penetrating ability in pancreatic tissues therefore; it is also used in local pancreatic infections [12]. The main issue with the use of this newer quinolones is lack of confidence of physicians and surgeons over prescribing Moxifloxacin in severe acute pancreatitis. This study may contribute in building the confidence level of physicians and surgeons in prescribing Moxifloxacin in SAP without any reluctance.

The aim of this research work was to study the in-vitro comparative activity of different concentrations of Moxifloxacin and Ciprofloxacin against the common key pathogens of SAP in cohort population (a defined population is selected for longitudinal assessment of exposure-outcome relations) and also to observe the sensitivity pattern of the isolated pathogens of SAP with increasing concentration of Moxifloxacin and Ciprofloxacin. SAP is still a life threatening disease and majority of deaths related to SAP are associated with complicated bacterial infections and inflammation of pancreas. SAP is a polymicrobial infection of gram positive and gram negative pathogens.

Materials and Methods

Collection of clinical isolates

Total 140 isolates of SAP were provided by Jinnah Post Graduate Medical Center (JPMC) and Civil Hospital Karachi (CHK), collected from the pancreatic tissues obtained surgically. Pathogens *E. coli* (n=54), *Pseudomonas aeruginosa* (n=44), *S. aureus* (n=28), and *Klebsiella* spp. (n=14) were isolated from patients between ages of 18 to 70 years.

In vitro studies

These organisms were grown and subculture on EMB agar and were further identified by bio-chemical tests such as Indole positive test for *E.coli*, Catalase positive test for *P. aeruginosa* Coagulase positive test for *S. aureus*, and Simmon citrate positive test for *Klebsiella*. The percentage resistance and sensitivity of various isolates of SAP using agar dilution method against moxifloxacin and ciprofloxacin were determined. The experimental work was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The sub grown cultures were then suspended in 1 ml Muller Hinton broth. After 4 hours of the incubation, the broth got turbid showed bacterial growth and was matched with 0.5 MacFarland turbidity standards which contain 1×108 CFU/ml (Colony forming unit). Series of different concentrations i.e. 0.5, 1, 2, 4, 8, and 16 mg/L for both drugs were prepared in double distilled water, then these concentrations were mixed with liquid agar medium at (45 to 50oC) in a ratio of 1:9 (one part of drug to 9 part of medium) and then this prepared solution was poured in to sterilized petri plates near flame and were allowed for solidification [13]. Petri dishes were arranged in order of increasing concentration of both drugs. Series of plates were prepared with addition of multiple inoculums by replicators [14]. After 24 hours of incubation, number of resistant and sensitive strains was observed and results were calculated, by ignoring single colony or growth [15-16].

Results

Results showed that *E.coli* has inverse relation against concentration of drugs i.e., resistance of E. coli decreased with increasing the concentration. From Table 1, it was observed that the average resistance of 54 isolates of *E. coli* showed 17% and 11% against ciprofloxacin and moxifloxacin, respectively.

Table 1. Resistance level of moxifloxacin and ciprofloxacin against *Escherichia coli* (n=54 isolates)

	Moxifloxacin						Ciprofloxacin					
Concentration (mg/L)	0.5	1	2	4	8	16	0.5	1	2	4	8	16
% age Resistant	66	61	50	37	22	11	72	67	57	41	30	17
% age Sensitive	34	39	50	63	78	89	28	33	43	59	70	83

This revealed that moxifloxacin was more effective against *E. coli* than Ciprofloxacin. In case of *Pseudomonas aeruginos*a moxifloxacin and ciprofloxacin displayed poor activity. *Pseudomonas aeruginosa* showed 48% and 50% resistance against moxifloxacin and ciprofloxacin, respectively after studying 44 isolates of SAP as shown in Table 2.

Table 2. Resistance level of moxifloxacin and ciprofloxacin against *Escherichia coli* (n=54 isolates)

	Moxifloxacin						Ciprofloxacin					
Concentration (mg/L)	0.5	1	2	4	8	16	0.5	1	2	4	8	16
% age Resistant	95	93	84	77	66	48	98	95	86	82	68	50
% age Sensitive	5	7	16	23	34	52	2	5	14	18	32	50

A total of 28 isolates of *S. aureus* were used in SAP and studied at similar maximum concentration of 16mg/L. An average 18% resistance was observed with Moxifloxacin while it increased to 39% for ciprofloxacin as shown in Table 3.

Table 3. Resistance level of moxifloxacin and ciprofloxacin against *S. aureus* (n=28 isolates)

	Moxifloxacin						Ciprofloxacin					
Concentration (mg/L)	0.5	1	2	4	8	16	0.5	1	2	4	8	16
% age Resistant	75	75	68	46	32	18	93	89	75	61	46	39
% age Sensitive	25	25	32	54	68	82	7	11	25	39	54	61

Klebsiella spp. was the most susceptible organism to both drugs. Only one isolate showed resistance to Moxifloxacin out of 14 strains which were about 7%. For Ciprofloxacin this resistance was 14% as mentioned in Table 4.

Table 4. Resistance level of moxifloxacin and ciprofloxacin against *Klebsiella* spp (n=14 isolates)

	Moxifloxacin						Ciprofloxacin					
Concentration (mg/L)	0.5	1	2	4	8	16	0.5	1	2	4	8	16
% age Resistant	86	86	79	57	36	7	93	86	86	71	50	14
% age Sensitive	14	14	21	43	64	93	7	14	14	29	50	86

The percentage resistance of all isolate against moxifloxacin and ciprofloxacin shown in Figure 1 and percentage susceptibility shown in Figure 2.

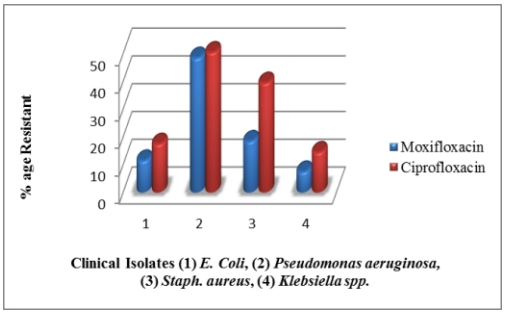

Figure1 : Percentage Resistance of *E. coli*, *Pseudomonas aeruginosa*, *Staph. aureus*, and *Klebsiella* spp. against both antibiotics.

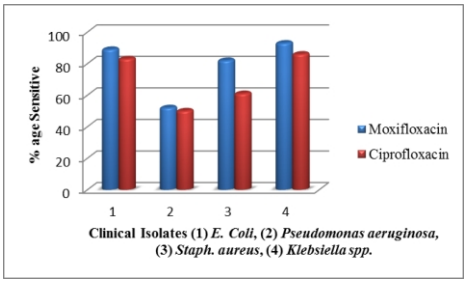

Figure 2 : Percentage Sensitivity of *E. coli*, *Pseudomonas aeruginosa*, *Staph. aureus*, and *Klebsiella* spp. against both antibiotics.

Discussion

SAP and the predominant gram negative and gram positive microorganisms were involved in this study. We found 39% strains of *E.coli* as the most prominent bacteria. *E.coli* is the most common

gram negative pathogen in pancreatic infections [2]. A number of other studies also prove *E.coli* as a most common pathogen. The second leading pathogen was *P. aeruginosa* and calculated as 31%. In a study of pancreatic infection in India, [17] also claimed *E.coli* and *Pseudomonas aeruginosa* as a most common pathogen which was isolated [17]. Whereas *S. aureus* and *Klebsiella* spp. isolates were calculated about 20% and 10% respectively. These pathogens create major challenge in the management of SAP, so we successfully calculated the resistance level of these pathogens of SAP against moxifloxacin and ciprofloxacin. Moxifloxacin showed significant and excellent activity against all pathogens of SAP as compare to ciprofloxacin. From Table 1, it can be concluded that *E.coli* was about 6% more resistant to ciprofloxacin than moxifloxacin. An average 18% resistance was observed with moxifloxacin while it increased to 39% for ciprofloxacin as shown in Table 3. In another in vitro study conducted for moxifloxacin, *S. aureus* showed 14% resistance out of n=206 strains [18]. In USA, a study was conducted in Creighton University School of Medicine and they found that 71% strain of *Klebsiella* spp. were susceptible to moxifloxacin and 79% to ciprofloxacin [19]. In case of *S. aureus*, this difference was about 21.43% while in *Klebsiella* spp. resistance against ciprofloxacin was about 2 fold greater than moxifloxacin. *Pseudomonas aeruginosa* was the only microorganism which was more resistant to both of the antibiotics as shown in Figure 1 but *Pseudomonas aeruginosa* showed 48% and 50% resistance against moxifloxacin and ciprofloxacin respectively after studying 44 isolates of SAP as shown in Table 2 which means that *Pseudomonas aeruginosa* is more resistant to moxifloxacin. The reason of this strong resistance of *Pseudomonas aeruginosa* against both drugs is may be due to misuse, overdose or inadequate use of quinolones and other antibiotics to which these pathogens acquired resistance. This pathogen might be sensitive to moxifloxacin and ciprofloxacin at concentration >16mg/L. Different concentrations of antibiotics were also influenced on resistance patterns i.e. at all concentration <16mg/L also inhibits bacterial growth, but the maximum inhibition was observed at maximum concentration of ≤16 mg/L.

The percent sensitivity of both drugs at different concentrations against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, and *Klebsiella* spp. is given in Figure 2. From Figure 2 it may be concluded that the isolates of SAP i.e., *E.coli, Staph aureus* and *Klebsiella* species were more susceptible to moxifloxacin than ciprofloxacin. The average sensitivity or susceptibility of *E. coli, S. aureus* and *Klebsiella* against moxifloxacin at16 mg/L was about 89%, 82% and 93%, while it was 83%, 61%, and 86% at similar concentration of ciprofloxacin. Both drugs display poor sensitivity against *Pseudomonas aeruginosa* and it was about 52% and 50%, respectively.

Conclusion

It is concluded that, the clinical isolates collected were susceptible against both the antibiotics but the resistance pattern is increasing in our population which is a threat in the future. Therefore, the physicians may prescribe these antibiotics unless no other substitute is available in clinical practice. Thus, the moxifloxacin may potentially be effective chemotherapy in the management of SAP and other complications of pancreas.

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Declaration of Interest

The authors do not have any conflict of interest

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