Short Communication

FIRST GENERATION AND FOURTH GENERATION CEPHALOSPORIN SUSCEPTIBILITY AGAINST CLINICAL ISOLATES

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ABSTRACT:

PURPOSE: Curing of infections is usually on the top priority of health associated-professionals. Resistance of microbes by antibiotics is worrying situation for medical professionals. Cephalosporins are one of the most commonly used β-lactam antibiotics. Cefepime and cephalexin are belonging to fourth and first generation cephalosporin respectively. The main aim of the present study is to evaluate the difference in resistance a pattern of pathogens between these two generations of anti-infective agents.

METHOD: During the present study, *in-vitro* antibacterial activity of cefepime and cephalexin has been observed by Kirby-Bauer method (disk diffusion method). A sum of 91 clinical isolates of *Staphylococcus aureus* (43), *Escherichia Coli* (37), and *Klebsiella pneumoniae* (11) have been isolated from urine, stool, sputum and surgical, burn, and accidental wound pus from different hospitals at Karachi.

RESULT: The present study has revealed that *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* has been shown 37.7%, 26.3%, and 34.8% resistance against cefepime, fourth generation cephalosporin respectively, while 77.4%, 91.7%, and 89.6% *S. aureus, E. coli*, and *K. pneumoniae* has been resistant against first generation cephalosporin, cephalexin.

CONCLUSION: It has concluded from the present study, that cefepime 4th generation cephalosporin is far much better susceptibility as compared to cephalexin. The study has been strongly recommended the routine monitoring and observation of susceptibility of microbes against respective antibiotics before administration.

KEYWORDS: Cephalosporin; cephalexin; cefepime; clinical isolates; sensitivity

INTRODUCTION:

Microorganisms are living in human body as commensalism and pathogens and also in food material by which the individual may suffer from infections.[1-4] Antibacterial activity of antibiotics has been chiefly depending upon the concentration of drug reached in systemic circulation and infected tissue. Microorganisms have the ability to transfer their resistance traits to the antibiotics to their next generation.[5] Resistance of pathogen has been developed against antibiotics due to low dose antibiotics, long course of antibiotics[6], too early and too late administration[7], suboptimal, irrational and extensive use.[8] Several workers have been found that low concentration of antibiotics is one of the significant aspects to increase the resistance against antibiotics.[9-11] Since last three decades, the use of broad spectrum antibiotics has been increased due to many factors like decreased drug interactions, and reduced the risk for toxicity, and pharmacoeconomic advantages.[12]

Cephalosporins, β-lactam antibiotics have better efficacy with tolerability as compared to other group of antibiotics. By cephalosporins, patients are not as much of hypersensitive with cephalosporins as compared to other β-

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lactams like penicillin and its derivates. Cephalosporin has been generally classified into four generations.[13]

Cephalexin is first generation empirical oral cephalosporin. Cephalexin has been prescribed as first line therapy for cellulitis, and patients sensitive to penicillin. Cephalexin has been indicated in the treatment of otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, and urinary tract infections. It may be used to prevent bacterial endocarditis and urinary tract infections (UTIs).[14] It has been mostly prescribed in USA in 2008. Cephalexin has been provided antimicrobial activity against Gram-positive bacteria like methicillin-susceptible *Staphylococcus aureus* (MSSA), coagulase-negative *Staphylococci*, penicillin-susceptible *Streptococcus pneumoniae*, and Gram-negative bacteria such as *Moraxella catarrhalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*. It has been mainly indicated in skin and soft tissue infections (SSTI), UTIs, and respiratory tract infections (RTIs) during pregnancy due its safety profile.[15]

Cefepime, a fourth generation cephalosporin has definite edge over the first generation cephalosporin. Cefepime has not only broad spectrum action but also acquired bactericidal activity against resistant Gram-positive pathogens like methicillin-resistance *Staphylococcus aureus* (MRSA), penicillin resistance *Streptococcus pneumoniae*, *Streptococcus pyogenes* and extended-spectrum β-lactamases (ESBLs), producing Gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia*, *Citrobacter*, *Proteus mirabilis* and less active against *Bacillus fragillis* and *Pseudomonas aeruginosa*.[16,17] Cefepime has been the drugs of choice in life threatening infections like febrile neutropenia, septicemia, and in severe infections of intensive care units (ICU) patients. Several workers have reported that cefepime has been less sensitive to extended-spectrum β-lactamases (ESBLs) as produced by Gram-negative pathogens than other generations of cephalosporin.[18]

MATERIALS AND METHODS:

COLLECTION OF SPECIMENS

A total of 91 clinical isolates belonging to different genera were isolated from urine, stool, sputum, surgical, burn, and accidental wound pus either mixed or single culture. *Staphylococcus aureus* (n=43)*, Escherichia coli* (n=37)*, and Klebsiella pneumoniae* (n=11) were collected on sterile swabs. Clinical isolates were obtained from patients who had developed sign and symptoms of infections from different hospitals at Karachi. The isolates were identified based on their colony characteristics on different media and confirmed by biochemical reactions. The isolates were inoculated in caso agar/ tryptic soya agar slants. The slants had been preserved at 4^{o}C in the refrigerator. Anti Microbial Resistance (AMR) has been determined by Clinical and Laboratory Standard Institute (CLSI, formally NCCLS) reference disk diffusion (Kirby-Bauer) method[19,20].

Table 1: Summary of Clinical Isolates

Clinical Isolates	Source of Clinical Isolates	Number of Clinical Isolates
Staphylococcus aureus	Surgical, Burn and Accidental wound pus (Skin and soft tissue infections)	43
Escherichia coli	Stool and Urine (Intra-abdominal and urinary tract infections)	37
Klebsiella pneumoniae	Surgical wound pus (Skin and soft tissue infections), Sputum (Respiratory tract infection)	11

Table 2: Resistance Pattern of Clinical Isolates Involved in Different Infections

Antibiotics	Clinical Isolates	Disc Diffusion Method†			Percentage of Resistance
		Sensitive	Intermediate	Resistance	
Cephalexin	*Staphylococcus aureus*	≥ 18	15-17	≤ 14	77.4%
	Escherichia coli	≥ 18	15-17	≤ 14	91.7%
	Klebsiella pneumoniae	≥ 18	15-17	≤ 14	89.6%
Cefepime	*Staphylococcus aureus*	≥ 18	15-17	≤ 14	37.7%
	Escherichia coli	≥ 18	15-17	≤ 14	26.3%
	Klebsiella pneumoniae	≥ 18	15-17	≤ 14	34.8%

PREPARATION OF INOCULUMS

Muller-Hilton Broth (MHB) was used to prepare inoculums and matched with McFarland standard. All tubes were incubated at 37 ^{o}C for few hours to develop the required turbidity as that of the McFarland standard. Muller- Hilton Agar (MHA) was used to determine the sensitivity of clinical isolates. Bauer, Kirby, Sherris and Tuck strongly suggested Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration.[19]

INOCULATION OF BACTERIAL CULTURE

A sterile swab was dipped into a broth suspension of bacterial culture. Excess inoculum was removed by rotating the swab against the inside wall of the tube with slim pressure. The whole surface of MHA plate was then streaked uniformly in three directions approximately at 60^{o} angle from each other. The lid was then replaced and the plates were allowed to dry for 10-15 min.

PLACEMENT OF ANTIBIOTIC DISC

The appropriate antibiotic impregnated discs were placed on the agar surface with sterile forceps. Each disc was pressed down gently with the forcep to assure good contact with agar surface. The disc should be distributed such that each is at least 24 mm from center to center of its nearer neighbor and 12 mm from the edge of plate.

INCUBATION

The plates were overturned within 15 min of placing the disc on agar and incubated at 35-37 ^{o}C for 24 hours. After incubation the diameter of the clear zones around the antibiotic disc were measured by using vernier caliper. All the bench work was carried out near a flame to create a zone of inhibition of invading bacteria and maintained the integrity.

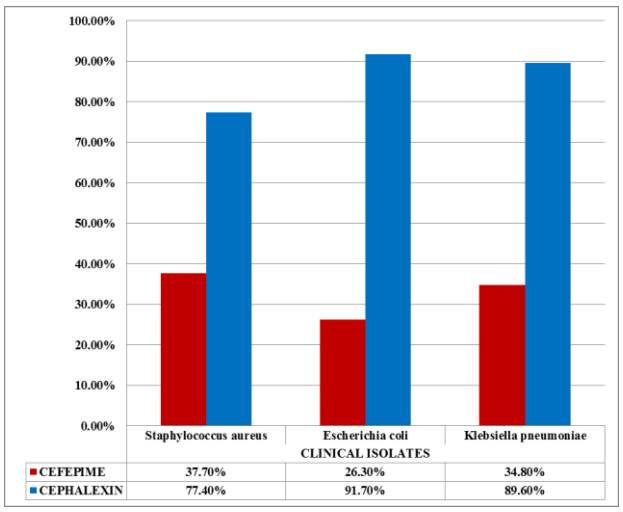
RESULT:

We have found total 91 clinical isolates from different hospital labs and pathological laboratories at Karachi. *Staphylococcus aureus* (n=43), the most isolated microbes, mainly from surgical, burn, and accidental wound pus samples, *Escherichia coli* (n=37) is the second competitor clinical isolate, mainly isolated from urine, sputum, and wound pus samples, while *Klebsiella pneumoniae* (n=11) has been least isolated pathogen, mostly isolated from sputum and urine samples. The summary of the source of clinical isolates has been given in table 1.

By the present study, it has been revealed that 37.7%, 34.8%, 26.3% resistance against *S. aureus*, *K. pneumoniae, E. coli* against cefepime, while cephalexin has possessed 77.4%, 89.6%, and 91.7% resistant to *S. aureus*, *K. pneumoniae, E. coli*. Cephalothin-class disc to predict cephalexin susceptibility discs was emphasized. Respective first and fourth generation cephalosporin have contained 30 µg content.[20] The outcome susceptibility of clinical isolates has been shown in table 2 and fig. 1.

DISCUSSION:

The main aim of study is to find the difference between resistances of microbes among 1st generation cephalosporin (cephalexin) and 4th generation cephalosporin (cefepime). It has been known mainly resistant virulent pathogens like MRSA and Extended-Spectrum Beta-lactamase (ESBL) producing bacteria like *Escherichia coli* and *Klebsiella pneumoniae*. It has been proven that the resistance against β-lactam has been increased.



In vitro study of antibacterial action has been performed frequently, as consequences of the sensitivity can be used to conclude how a drug would act inside the body.[21] By the prolong use of antibiotics, resistance of pathogenic bacteria against antibiotics has been a main concerned. Resistance of antibiotics is also on priority not only for health-associated professionals but also a key problem for pharmaceutical industry. The illogical prescription of antibiotics has increased the chances of resistance. Moreover, a huge investment of time and money of pharma-industries has been lost due to irrational use of antibiotics.[4,22]

Cephalosporins are one of the most commonly used belonging to β-lactam class of antibiotics. Till now established four generation of cephalosporins are used by physicians and marketed by pharmaceutical industries. Due to its better tolerability, durability, and excellent pharmacokinetic profile, it is one of the most prescribed antibiotics. Several pathogens have been developed β-lactamase which aid in the resistance against bactericidal activity of cephalosporins. Some novel broad spectrum cephalosporins have been soon introduced in market like ceftaroline[23] and ceftobiprole[24] for the prevention and cure of virulent resistant bacteria. They also have an edge over penicillin, as 10% population is sensitive to cephalosporins in 100% penicillin hypersensitive persons.

Cephalexin, first generation oral cephalosporin has been marketed by Eli Lilly in 1967. It has been widely prescribed in otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulitis, and UTIs. The gram-negative bacilli, *Escherichia coli, Klebsiella pneumoniae* have been less susceptible to cephalexin than the gram-positive cocci, the resistance of *E. coli* (91.7%) and *K. pneumoniae* (89.6%) has been shown disappointing position.[25] As, it has been found by present study cephaexin has been resistant by methacillin-sensitive *S. aureus* (MSSA) around 78%. The present study has been supported by Anupurba *et al*.[26] In 1981, Bayston and Swinden[25] reported 56% resistant of methacillin-resistant *S. aureues* (MRSA) against cephalexin. In 2008, cefalexin was the most popular cephalosporin antibiotic in the United States. The increased in resistant of microbes against cephalexin has been developed due to irrational use.

Cefipime, a fourth generation cephalosporin developed in 1994. It has been usually prescribed in moderate-severe nosocomial pneumonia, infections caused by multi-resistant microorganisms (e.g. *P. aeruginosa*; MRSA) and empirical treatment of febrile neutropenia.[27] The present study has been supported by Nasiri *et al*.[28] The outcome of resistance of *S. aureus* was 37.7% in present study. In Taiwan, Liao *et al*. have reported 33% resistance of *E. coli* against cephalexin.[29] 89.6% resistance of *K. pneumoniae* against cefepime has been found by our study. Winokur and co-workers have also been reported the resistance of *K. pneumoniae* against cefepime.[30]

As it has been observed, the resistance of microbes even against 4th generation cephalosporin (cefipime) has been increased

with the span of time due to irrational use. The treatment of infectious disease is one of the most troubled issue for health concerned professionals and main cause of morbidity and mortality. In underdeveloped countries there is lacking of the usage of antibiotics guidelines.

CONCLUSION:

The present study has suggested the health associated professional to follow the guidelines. Moreover, there must a national guideline for health associated professionals to obstruct the illogical use of antibiotics. The antibiotics should be only sale on prescriptions. The culture sensitivity should be promoted to reduce the use of newly broad spectrum antibiotics. The pharma-industries should be promoted their antibiotics in a specific indication to increase the life of susceptibility of their antibiotics. It has been suggested that local bodies should prepared guidelines for initial treatment.

Due to financial crisis in underdeveloped countries, there is also a deficiency of local surveillance program. Due to implacable disobedience of the frequent use of antibiotics in underdeveloped countries, the world renowned regulatory bodies like WHO, NICE, CDC, and several infection control society were steadily recommended the day to day inspection plan to control the misuse of antibiotics. The regulatory bodies also force physician to strictly fellow the guidelines of empiric therapy to avoid the resistance and to tend and treat infections.

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