JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Comparative Study on Resistance Pattern of Staphylococcus *Aureus* against Amoxicillin, Cefaclor, Levofloxacin and Tetracycline

Sonia Ayub*, Bilqees Fatima, Syed Baqir Shyum Naqvi, Dilnawaz Sheikh, Syed Muzaffar Ali [1], Faiza Ayub[2]

1 Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University Karachi-74600, Pakistan

2 Jinnah Medical and Dental Collage 22-23 Shaheed-i-Milat Road Karachi

ABSTRACT:

Present investigation was undertaken to evaluate the susceptibility and resistance pattern of Staphylococcus aureus causing different types of wound infections and to compare the efficacy of antibiotics namely Amoxicillin, Cefaclor, Levofloxacin and Tetracycline. The in-vitro antibacterial activity and resistance patterns of these four well known antibiotics were studied and compared by using disk diffusion method. For this, one hundred and three clinical isolates of, Staphylococcus aureus (103) and information regarding patient age, sex and bacterial organism isolation were collected from different local pathological laboratories and hospitals according to the zones (east Karachi, west Karachi, south Karachi and north Karachi) of Karachi (Pakistan) during the time period of February 2015 to June 2015. Out of the sample analyzed, resistant pattern of one hundred and three (103) clinical isolates 99 (96.1%) sample exhibited resistance against amoxicillin. While, 89 (86.4%) and71 (68.9%) sample showed sensitivity, against Cefaclor and levofloxacin respectively .while, 84 (81.6%) sample of Staphylococcus aureus were sensitive against tetracycline. The study showed that the antibacterial activity of cefaclor is more as compare to amoxicillin. Amoxicillin is not the first choice to treat the infections against Staphylococcus aureus because they showed resistance 96.1%. Cefaclor is the first choice to treat infection which is caused by Staphylococcus aureus because they showed 86% sensitivity against Staphylococcus aureus. The prevalence rate of infection which is caused by Staphylococcus aureus is more common in female.

KEY WORDS: Staphylococcus aureus, Amoxicillin, Cefaclor, Levofloxacin, tetracycline

Article history:

Received 12 Aug 2015
Revised 15 Aug 2015
Accepted 26 Aug 2015
Available online 01 Nov 2015

Citation: Ayub S., Fatima B., Naqvi S. B. S, Sheikh D. Ali S. M., Ayub F. Comparative Study on Resistance Pattern of Staphylococcus Aureus against Amoxicillin, Cefaclor, Levofloxacin and Tetracycline. **J Pharm Sci Bioscientific Res**. 2015 5(6):594-599

***For Correspondence:* Ms. Sonia Ayub**

Research Scholar, Department of Pharmaceutics, Hamdard university, Karachi, Pakistan

INTRODUCTION:

During the past decades, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes but these bacteria and microbes are remarkably robust and have developed several ways to resist antibiotics. This problem is due to increasing use, and misuse, of the existing antibiotics in human being and animals [1]. Antibiotic agents are among the most important contributors to the modernization of medicine, and it is difficult to imagine the continuation of advances of recent years without them. [2] Antibiotics are active against number of micro-organism however, antibiotics did not kill or retard the growth of all types of microbes because micro-organism resistance increases day by day some of the microbes are naturally resistant and some obtain resistance by changing in their structure, altering the permeability & produce the enzyme which inactivates the antibiotics activity. [3] *Staphylococcus aureus* poly-microbial wound infections is of great importance due to their pervasive nature,

increasing occurrence, growing resistance to antimicrobial agents, and ability to delay healing. Methicillin-resistant S. aureus USA300 is the leading cause of community-associated bacterial infections resulting in increased morbidity and mortality. [4] Well known resistance carrier with high clinical impact include the Gram-positive organisms *Staphylococcus aureus* [5]. The quinolones are synthetic, chemotherapeutics, broad-spectrum antibiotics. Quinolones has shown their activity against Gram- positive and Gram-negative bacteria. [6] Tetracycline is a broad spectrum antibiotics cover Gram-negative and Gram-positive bacteria and other species and widely used in human and veterinary medicine [7][8]. Tetracycline analogues oxy-tetracycline, tetracycline hydrochloride and dimethyl-chlortetracycline developed and use against infections started in early 1950s. [9][10] Cefaclor inhibits the cell wall synthesis of bacteria [11] [12]. Cefaclor had greater antibacterial activity than either cephalexin or cephradine against isolates of *Staphylococcus aureus & Klebsiella pneumoniae*, [13]

The objective of the present work was to determine the resistance pattern according to age, sex and location of one hundred and three clinical isolates of *Staphylococcus aureus* against four antibiotics namely amoxicillin, cefaclor, levofloxacin and tetracycline by using Bauer - Kirby method. These four antibiotics have wider spectrum of activity against bacteria. They were selected for their wider use by the physician

METHODS AND MATERIALS:

Collection of clinical isolates

One hundred and three clinical isolates were procured from different pathological laboratories of Karachi city during the time period of February 2015 to June 2015.

Isolation and purification

All specimens were inoculated on 5% blood agar, MacConkey agar and Chocolate agar plates and incubated overnight at 37 °C aerobically. Preliminary identification of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Bacterial pathogens were identified by conventional Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method recommended by the National committee for clinical laboratory standards (NCCLS).

Antimicrobial agents

Standard discs of amoxicillin, Cefaclor, levofloxacin and tetracycline were procured from market. Cartridges containing discs were stored in refrigerator (2◦C to 8◦C).

Preparation of media

Mueller Hinton Agar and Mueller Hinton Broth were prepared and sterilized according to manufacturer's instructions (Merck).

Preparation of media plates

Mueller Hinton Agar plates were prepared for this research task.

Preparation of Inoculum:

The inoculation was prepared by touching the top of the colonies of the isolates with sterile wire loop and suspending in a tube containing 4-5 ml of broth and incubated at 37°C for 4-6 hours. [16]

Inoculation of plates:

Sterile swab was dipped into inoculum suspension. Excess fluid was removed by pressing and rotating the swab against the side of tube above the level of suspension. The swab was then spell evenly over the surface of the medium in three directions, rotating the plates approximately 60 degree to ensure even distribution. After inoculation, surface of agar was allowed to dry for 5 minutes. McFarland standards were prepared by mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. For example, A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1% barium chloride, dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO4). The cell density /concentration was approx 1.5X10^8 CFU/mL while % Transmittance at wavelength of 600 nm was 74.3 and Absorbance was 0.132. [2]

Placement of antibiotic disc:

Using sterile forceps, the appropriate antimicrobial discs of amoxicillin, Cefaclor, levofloxacin and tetracycline

were placed on the agar surface and slightly pressed down to ensure rigid.

Incubation of Plates:

Within 30 minutes of applying the discs, plates were incubated at 37ºC in incubator for 18-24 hours.

Measurement of Zone diameter and interpretation of result:

After 24 hours of incubation, the plates were examined and zone of inhibition was measured (in mm) with the help of Vernier caliper which were shown in tables, graphs and figures.

RESULTS

In the present study, resistant pattern of one hundred and three (103) clinical isolates of *Staphylococcus aureus* were studied by using amoxicillin, cefaclor, levofloxacin and tetracycline the results are presented in graph 1-4. Data analysis was done by using SPSS version 20 and the results were manipulated according to the Clinical and Laboratory Standards institute (CLSI) 2011.

Table1: Clinical and Laboratory Standards institute (CLSI) 2011 guideline of antimicrobials

CLSI standards for Zone Diameter of *Staphylococcus aureus*				
Antimicrobial agent	**Disk content**	**Break points, Nearest whole mm**		
		S	**R**	**I**
Amoxicillin	10µg /20µg	≥ 20	–	≤19
Cefaclor	30µg	≥ 18	15-17	≤14
Tetracycline	30µg	≥ 19	15-18	≤14
Levofloxacin	5µg	≥ 19	16-18	≤15

Among 103 samples of *Staphylococcus aureus* 4(3.9%) sample showed sensitivity, no clinical isolates showed intermediate response and 99 (96.1%) sample exhibited resistance against amoxicillin. While, 89 (86.4%) sample showed sensitivity, 4(39%) showed intermediate response and 10 (9.7%) sample exhibited resistance against cefaclor and 71 (68.9%) sample of *Staphylococcus aureus* were sensitive against levofloxacin, 9 (8.7%) showed intermediate response and 23(22.3%) were resistant to the levofloxacin 5 µg. while, 84 (81.6%) sample of *Staphylococcus aureus* were sensitive against tetracycline, 6(5.8%) showed intermediate response and 13 (12.6%) were resistant to the tetracycline 30 µg. 32 (31.1%) samples were collected from children, 43(41.7%) from female and 28 (27.2%) from the male patient. Clinical isolates collected according to the age, 32 samples collected from the age between 1-12 years, 22 from 13-24 years, 30 from 25-36 years, 7 from the age limit between 37-48 years,7 from 49-60 years and 5 sample were collected from the age of 61-72 years. Patient data and sample were collected according to the zones of Karachi, 37 (35.9%) from north, 6(5.8%) from west, 21(20.4%) from east and 39(37.9%) from south Karachi. 87(84.5%) clinical isolates were collected from pus, 3(2.9%) from wound swab ,4 (3.9%) from left ear swab,1 (1.0%) from throat swab, 3 (2.9%) from urine ,2 (1.9%) from catheter tip,2 (1.9%) from blood and 1(1.0%) from right ear swab.

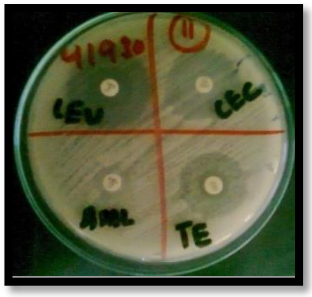


Figure 1: antibacterial activity of amoxicillin, cefaclor, levofloxacin and tetracycline against *Staphylococcus aureus* by using Kirby –Bauer Method

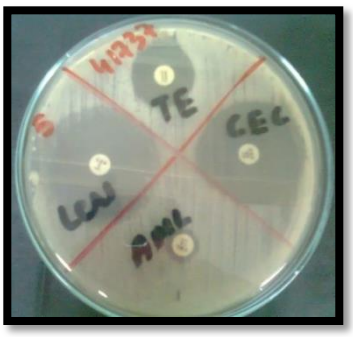
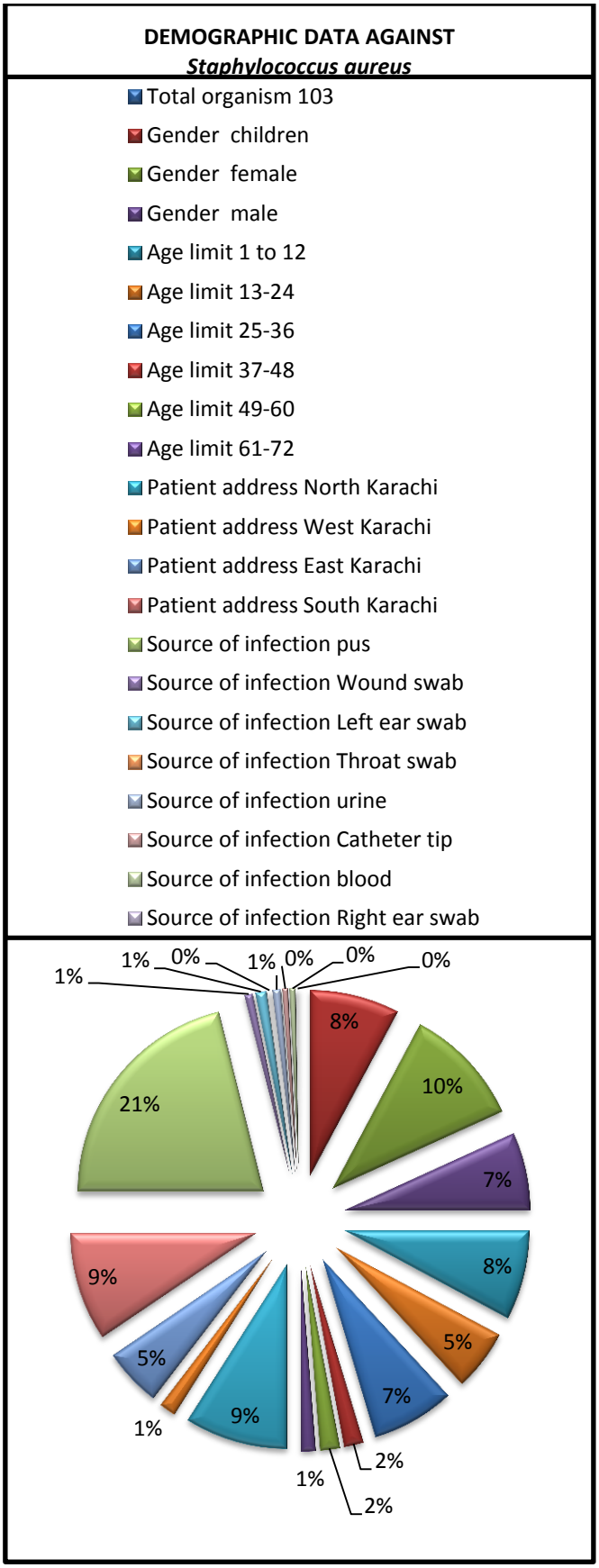


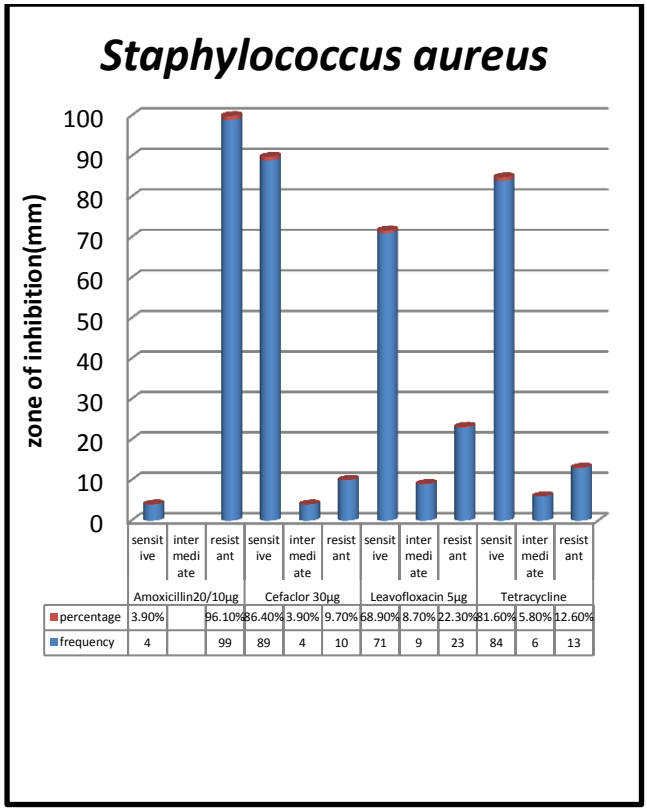
Figure 2: antibacterial activity of amoxicillin, cefaclor, levofloxacin and tetracycline against *Staphylococcus aureus* by using Kirby –Bauer Method

Table 2: Demographic data of the patients

Total organism	103	frequency
Gender	children	32
	female	43
	male	28
Age limit	1-12	32
	13-24	22
	25-36	30
	37-48	07
	49-60	07
	61-72	05
Patient address	North Karachi	37
	West Karachi	06
	East Karachi	21
	South Karachi	39
Source of infection	pus	87
	Wound swab	03
	Left ear swab	04
	Throat swab	01
	urine	03
	Catheter tip	02
	blood	02
	Right ear swab	01



Graph 1: graphical representation of demographic data of the patients



Graph 2: Antimicrobial sensitivity pattern of *Staphylococcus aureus* against amoxicillin, Cefaclor, levofloxacin and tetracycline

DISCUSSION:

During this study four antimicrobial agents i.e. amoxicillin, cefaclor, levofloxacin and tetracycline were used against 103 clinical isolates of *Staphylococcus aureus.* Amoxicillin showed more resistance then levofloxacin, tetracycline and cefaclor. Amoxicillin showed 96% resistance against *Staphylococcus aureus* so; amoxicillin is not the first choice to treat infection which is due to the *Staphylococcus aureus.* Cefaclor is the first choice to treat infection because it showed 86% sensitivity against *Staphylococcus aureus*. All these antibiotics have wider range of antibacterial activity against *Staphylococcus aureus* but this activity is not 100%. This is an alarming situation, so it is very important that the drugs should be prescribed under condition which is related to the particular infections unless other alternate is not available.

The prevalence rate of infection which is caused by *Staphylococcus aureus is* more common in female. in comparison with children and male patients and at the age between 1 to 12 year and 25 to 36 year and most of the clinical isolates were collected from south zone which is alarming situation government must take precautions and some serious steps to handle with this organism.

Multiple surveillance studies have demonstrated that resistance among prevalent pathogens is increasing at an alarming rate, leading to greater patient morbidity and mortality from nosocomial infections. Among Gram-positive organisms, the most important resistant pathogens are methicillin- (oxacillin-) resistant *Staphylococcus aureus*, *Escherichia coli, and Proteus mirabilis* [14].

A total of 257 clinical isolates were collected from different hospitals in Karachi and evaluated by using fifteen antibiotics belonging to different groups. *Staphylococcus aureus* (n=87), *Escherichia coli* (n=76), *Pseudomonas aeruginosa* (n=56), *Proteus* (n=21) and *Klebsiella* (n=17) species are the most common clinical isolates of surgical site infections. Among the semi-synthetic penicillins, ampicillin was found to be resistant to nearly all clinical isolates but amoxicillin was moderately sensitive to *Staphylococcus aureus*. Combinations of semi-synthetic penicillins are more sensitive than the penicillin alone. Co-amoxiclave exhibits superior sensitivity to all the surgical infection isolates *except Pseudomonas aeruginosa* which showed 68.75% resistance. *Pseudomonas aeruginosa* was highly resistant to cephalosporin except ceftraixone which showed 21.88% resistance. *Staphylococcus aureus* was slightly responsive to cefazolin, cephradine, cefaclor, ceftizoxime, cefuroxime and ceftriaxone [15].

This study revealed that clinical isolates collected from different pathological laboratories and hospitals of Karachi were susceptible to all these four antibiotics. Antibiotics resistance is due to increasing use, and haphazard of existing antibiotics in human therapy. The extensive use of antibiotics has resulted in bacteria rapidly developing resistance to these agents.

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