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Original Article

AMOXICILLIN AND TETRACYCLINE ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* AND *PROTEUS MIRABILIS*

SONIA AYUB*, BILQEES FATIMA, SYED BAQIR, SHYUM NAQVI, DILNAWAZ SHEIKH, SYED MUZAFFAR ALI, SHEIKH ABDUL KHALIQ[1]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University Karachi 74600, Pakistan
Email: soniaayub43@gmail.com

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ABSTRACT

Objective: Present investigation was undertaken to evaluate the susceptibility and resistance pattern of clinical isolates causing different types of infections and to compare the efficacy of antibiotics namely amoxicillin and tetracycline.

Methods: The in-vitro antibacterial activity and resistance patterns of these two well known antibiotics were studied and compared by using the disk diffusion method. For this, one hundred and thirty four clinical isolates comprising of, *Staphylococcus aureus (103)* and *Proteus mirabilis (31*) and information were taken 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.

Results: Out of the sample analyzed, resistant pattern of one hundred and thirty four (134) clinical isolates of *Staphylococcus aureus (103) and Proteus mirabilis (31)* were studied by using amoxicillin and tetracycline and the results are 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, 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 while, 2 (6.5%) clinical isolates showed sensitivity towards *Proteus mirabilis*, 19(61.31%) showed resistance and 10 (32.3%) showed intermediate resistance against amoxicillin. On the other hand, tetracycline showed 3(9.7 %) resistance against *Proteus mirabilis*, 26(83.9%) showed sensitivity and 2 (6.5%) clinical isolates showed intermediate resistance.

Conclusion: overall results of the present study showed that the antibacterial activity of tetracycline is more as compare to amoxicillin. Amoxicillin is not the first choice to treat the infections against *Proteus mirabilis* and *Staphylococcus aureus* because they showed resistance 96.1% and 61.3% against *Proteus mirabilis* as compare to tetracycline is the first choice to treat infection which is caused by *Staphylococcus aureus and Proteus mirabilis* because they showed 81.6% sensitivity against *Staphylococcus aureus and 83.9% sensitive against tetracycline.*

Keywords: *Staphylococcus aureus*, *Proteus mirabilis*, Amoxicillin, Tetracycline.

INTRODUCTION

Antibiotic agents are among the most important contributors to the upgrading of medicine, and it is difficult to visualize the continuation of advances of recent years without them [1, 2]. Antibiotics have been significant in the fight against infectious disease caused by bacteria and other microbes but these bacteria and microbes are surprisingly resilient and have developed several ways to resist antibiotics [3]. Bactericidal activity of antibiotics is mainly depends upon the concentration of drug in plasma and tissue [4]. Well known resistance carrier with high clinical impact includes the Gram-positive organisms *Staphylococcus aureus* and *Enterococcus* species [5] tetracycline is a broad spectrum antibiotics cover *Gram-negative* and *Gram-positive* bacteria and other species and widely used in human and veterinary medicine [6, 7]. Tetracycline analogues oxy-tetracycline, tetracycline hydrochloride and demethylchlortetracycline developed and use against infections started in early 1950s [8, 9]. *Proteus* species (spp.) are among the commonly implicated pathogens in hospitals as well as a cause of community acquired infections. Among the inpatients infection rate was 81.18% and out-patients 18.82 %. All the *Proteus* species were resistant amoxicillin-clavulanic acid, ceftazidime, and cefepime. The objective of the present work was to determine the resistance pattern of one hundred and thirty four clinical isolates comprising of *Staphylococcus aureus and Proteusmirabilis* against two antibiotics namely amoxicillin and tetracycline by using Bauer-Kirby method [10].

MATERIALS AND METHODS

Collection of clinical isolates

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

Antimicrobial agents

Standard discs of amoxicillin 20/10µg and tetracycline 30 µg were procured from the market. Cartridges containing discs were stored in the 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 the sterile wire loop and suspending in a tube containing 4-5 ml of broth and incubated at 37 °C for 4-6 h [11].

Inoculation of plates

Sterile swab was dipped into an inoculum suspension. Excess fluid was removed by pressing and rotating the swab against the side of the 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 min. 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_2SO_4). The cell

density/concentration was approx 1.5X10^8 CFU/ml while % Transmittance at the wavelength of 600 nm was 74.3 and Absorbance was 0.132 [3].

Placement of antibiotic disc

Using sterile forceps, the appropriate antimicrobial discs of amoxicillin and tetracycline were placed on the agar surface and slightly pressed down to ensure firm.

Incubation of plates

Within 30 min of applying the discs, plates were incubated at 37°C in incubator for 18-24 h.

Measurement of zone diameter and interpretation of result

After 24 h of incubation, the plates were examined and the zone of inhibition was measured (in mm) with the help of Vernier calliper which were shown in tables 1 to 2.

RESULTS

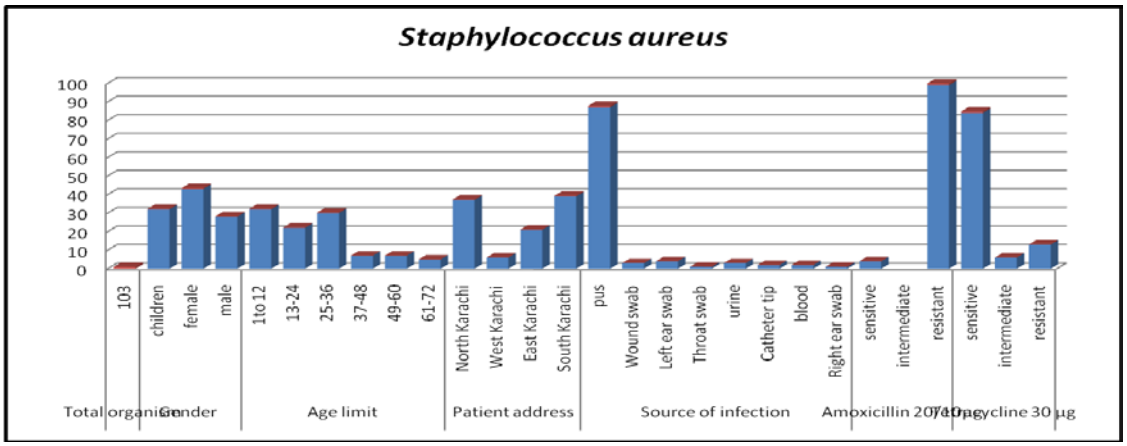
In our study, resistant pattern of one hundred and thirty four (134) clinical isolates of *Staphylococcus aureus (103) and Proteus mirabilis (31)* were studied using amoxicillin and tetracycline and the results are presented in tables 1-2 and graph 1-2. Data analysis was done by using SPSS version 20 and the results were manipulated according to the Clinical and Laboratory Standards institute (CLSI) 2011.

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, 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 y, 22 from 13-24 y, 30 from 25-36 y, 7 from the age limit between 37-48 y,7 from 49-60 y and 5 sample were collected from the age of 61-72 y.

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.

Table 1: Resistance pattern of Amoxicillin and Tetracycline against *Staphylococcus aureus*

Total organism	**103**		**100%**
Gender	children	32	31.1%
	female	43	41.7%
	male	28	27.2%
Age limit	1-12	32	31.1%
	13-24	22	21.4%
	25-36	30	29.1%
	37-48	07	6.8%
	49-60	07	6.8%
	61-72	05	4.9%
Patient address	North Karachi	37	35.9%
	West Karachi	06	5.8%
	East Karachi	21	20.4%
	South Karachi	39	37.9%
Source of infection	pus	87	84.5%
	Wound swab	03	2.9%
	Left ear swab	04	3.9%
	Throat swab	01	1.0%
	urine	03	2.9%
	Catheter tip	02	1.9%
	blood	02	1.9%
	Right ear swab	01	1.0%
Amoxicillin 20/10µg	sensitive	04	3.9%
	intermediate	—	—
	resistant	99	96.1%
Tetracycline 30 µg	sensitive	84	81.6%
	intermediate	06	5.8%
	resistant	13	12.6%

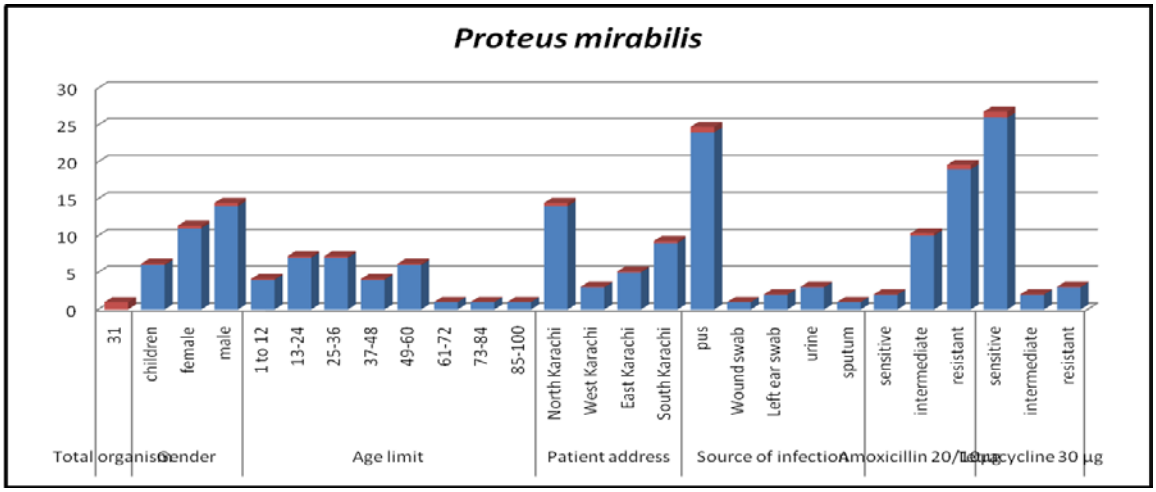


Graph 1: Percentages of *Staphylococcus aureus* against amoxicillin and tetracycline

31 sample of *Proteus mirabilis* were collected from the different zones of Karachi, from north Karachi 14(45.2%) clinical isolates were collected, 3 (9.7%) from west, 9 (29.0%) from south and 5 from the east Karachi (6.1%).2 (6.5%) clinical isolates showed sensitivity towards *Proteus mirabilis*, 19 (61.31%) showed resistance and 10 (32.3%) showed intermediate resistance against amoxicillin. On the other hand, tetracycline showed 3(9.7 %) resistance against *Proteus mirabilis*, 26 (83.9%) showed sensitivity and 2 (6.5%) clinical isolates showed intermediate resistance. 6 (19.4%) clinical isolates were collected from children from female 11(35.5%) and from the male 14(45.2%) clinical isolates were collected. The clinical isolates collected from pus were 24(77.4%) from wound swab 1(3.2%), 2 (6.5%) from left ear swab, 3(9.7%) from urine and 1 (3.2%) from sputum. 4 (12.9%) clinical isolates were collected from the age between 1-12 y, 7 (22.6%) from 13-24 y, 7 (22.6%) from 25-36 y, 4 (12.9%) clinical isolates from the age between 37-48, 6 (19.4%) from the 49-60 age,1 (3.2%) from 61-72, 1(3.2%) from 73-84 age and 1 (3.2%) from the age between 85-100 y.

Table 2: Resistance pattern of amoxicillin and tetracycline against *Proteus mirabilis*

Total organism	**31**		**100%**
Gender	children	06	19.4%
	female	11	35.5%
	male	14	45.2%
Age limit	1-12	04	12.9%
	13-24	07	22.6%
	25-36	07	22.6%
	37-48	04	12.9%
	49-60	06	19.4%
	61-72	01	3.2%
	73-84	01	3.2%
	85-100	01	3.2%
Patient address	North Karachi	14	45.2%
	West Karachi	03	9.7%
	East Karachi	05	16.1%
	South Karachi	09	29.0%
Source of infection	pus	24	77.4%
	Wound swab	01	3.2%
	Left ear swab	02	6.5%
	urine	03	9.7%
	sputum	01	3.2%
Amoxicillin 20/10µg	sensitive	02	6.5%
	intermediate	10	32.3%
	resistant	19	61.3%
Tetracycline 30 µg	sensitive	26	83.9%
	intermediate	02	6.5%
	resistant	03	9.7%



Graph 2: Percentages of *Proteus mirabilis* against amoxicillin and tetracycline

CONCLUSION

During this study two antimicrobial agents i.e. Amoxicillin and tetracycline, were used against 134 clinical isolates of *Staphylococcus aureus* and *Proteus mirabilis*. Over all results of the present study showed that the antibacterial activity of tetracycline is more as compare to amoxicillin. Amoxicillin is not the first choice to treat infections against *Proteus mirabilis* and *Staphylococcus aureus* because the showed resistance 61.3% and 96.1% respectively the antibacterial activity of tetracycline is more as compare to amoxicillin.

Tetracycline is the first choice to treat infection which is caused by *Staphylococcus aureus and Proteus mirabilis* because they showed 81.6% sensitivity against *Staphylococcus aureus* and 83.9% sensitive against tetracycline.

This study revealed that clinical isolates collected from different pathological laboratories and hospitals of Karachi were susceptible to both antibiotics. Antibiotic 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.

CONFLICT OF INTERESTS

Declared None

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