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ORIGINAL ARTICLE

Prevalence of Antibiotic Resistant Bacteria among the Clinical Samples from Different Hospitals of Peshawar, Pakistan

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ABSTRACT

Background: Majority of the microorganisms are responsible for causing diseases which can be fatal if left untreated. In Pakistan this might be very critical because of the misuse and/or improper use of antibiotics.

Objective: The current study was designed to point out the challenges of antibiotic resistance in Peshawar Pakistan.

Methods: A total of 100 samples, 25 each from blood, pus, skin, urine were collected from four different hospitals of Peshawar, Pakistan. The samples were grown on culture media after collection.

Results: Out 100 samples, 46 samples showed growth on culture medium. Four main isolates namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. *S. aureus* and *E. coli* were found in all tested samples blood, pus, skin, urine. *P. aeruginosa* was found in pus and *K. pneumonia* in urine only. The identified strains were subjected to sensitivity testing against 7 different antibiotics i.e., ampicillin, piperacillin tazobactam, doxycycline, aztreonam, ciprofloxacin, levofloxacin and teicoplanin.

Conclusion: All of the bacterial species were found resistant against the applied antibiotics except aztreonam.

Keywords: Prevalence, antibiotics sensitivity, *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*

INTRODUCTION

Microorganisms play a key role in spreading of many diseases and some outbreaks also took place due to these bacteria. In Pakistan, many individuals have been reported that are infected with *Tubercle bacilli*. Some antibiotic-resistant strains and chronic cases have also been reported [1]**.** In some studies extreme drug resistant (XDR) strains have been isolated and their genotypes were studied, the increasing threat of antibiotic resistance strain and XDR in Pakistan is due to inadequate detection and treatment [2]**.** Multidrug-resistant tuberculosis is reported in Pakistan with approximately 297,000 cases diagnosed per year [3].

In past several decades we have seen a remarkable increase in development of antibiotic resistance which leds to the discovery of extensively antibiotic resistant strains (XDR) and multidrug resistant strains (MDR) [4]. Adjacently, the field of life sciences especially the antibiotic resistance bacteria has gained considerable attention [5].When penicillin was unable to inhibit bacterial growth, it led to the discovery of the first beta-lactamase in bacterial strains [6]. Striking increase in development of beta-lactamase enzymes was observed of *staphylococci* [7]. In early 1960's the first beta-lactamase plasmids medicated strain was discovered and isolated from *Escherichia coli* found in blood sample of patient [8, 9].

Staphylococci is a major group of gram positive, non-motile, catalase and coagulase positive anaerobes which are involved in wide range of infections mainly nosocomial infections and also antibiotic resistance [10]. *Escherichia coli* is a pathogen commonly linked with community-associated and also with hospital-associated Infections. In past few years, it was reported that *E. coli* strains showed broad-spectrum resistance to antimicrobial agents. The emergence of pathogenic bacterial resistance to antibiotics became a major threat to general public health [11].

Sensitive bacteria can gain resistance through mutation or transfer of resistance genes located on mobile DNA elements known as integrons [12]. In Pakistan the increasing threat of antibiotic resistance strain and extensively drug resistant (XDR) is due to inadequate detection and treatment [13].

As antibiotic resistant strains are increasingly causing damage of humans and these strains are really hard to handle, this study is mainly focused on the isolation of antibiotic resistant strains from different clinical samples, such as urine, blood, wound, pus and many other sources, patients arrive to hospitals complaining about the problem which is unable to treat on locally available antibiotics and the problem was persistent, this was really a big problem and it was thought that infection could be because of viruses or fungi but most interestingly when samples were inoculated on different agar media's bacterial growth was found and when it was checked against certain antibiotics it was found that bacteria acquired resistance against locally available antibiotics. Which provided a base for conducting this study to find out bacterial resistance to locally available antibiotics.

MATERIALS AND METHODS

The samples were collected from different hospitals of Peshawar and brought to laboratory in sterile cotton swab. Samples were streaked on LB (Luria Bertani) agar plates and incubated at 37 ºC for 24 hours [14]. The samples were streaked on simple LB agar plates and incubated at 37 ºC for 24 hours and plates were then observed after 24 hours of incubation. Growth was observed on plates [14].

For identification of samples the isolated colonies were inoculated on differential media, such as blood agar medium and also on MacConkey agar medium. After inoculation, the plates were incubated at 37 ºC for 24 hours. These colonies were observed with light microscopy and further subjected to other biochemical tests. These tests contained catalase test, oxidase test, and indole test by the addition of Kovac's reagent, urease test, motility test and disc sensitivity test [14].

Catalase test was carried out in order to see the isolates were catalase positive or negative. Catalase test was mainly used for gram-positive strains because almost all Gram-negative strains were catalase positive so there was no need to carry out catalase test for gram-negative bacteria, however some members of *Enterobacteraceae* were divided into weakly positive and negative bacteria [14].

Oxidase test was carried out in order to find whether a microbe can oxidize certain aromatic amine or may not to form colored end products on filter paper [14].

Indole test was carried out to determine the ability of microorganism to oxidize tryptophan into Indole which indicated

the presence of tryptophanase enzyme which hydrolyze tryptophan into Indole, pyruvic acid and ammonium. The indole production in the reaction was detected by the addition of Kovac's reagent to the test tubes having bacterial cultures in Tryptone broth media [14].

Urease test was used to determine the production of urease enzyme by pure isolates. Urease is an enzyme that attacks the amide bond of the compound such as urea. In this reaction ammonia was liberated. Phenol red was used as indicator in the reaction and color change from yellow to red occurs. The change of color was due to the presence of urease enzyme [14].

Motility test was done for identification of samples. The motility test medium contained (TTC) triphenyletrazolium chloride, the samples were inoculated into fresh culture and then incubated at 37 ^{0}C for 48 hours. The result of motility test is very difficult to interpret, however the turbidity in the tubes indicated the motility of lactic acid bacteria [14].

Disc sensitivity test was used to find out the resistant strains of bacteria towards different antibiotics, nutrient agar plates were prepared and bacterial was inoculated in broth in test tube and samples were poured on plates for inoculation purpose. Nutrient agar plates were left for 15 mints and then antibiotics disc were placed on plate at the distance of inch from each other. Nutrient agar plates were incubated for 24 hours at 37 ^{0}C, after incubation plates were observed and results were noted [14].

RESULTS

A total number of collected samples was 100 out of which only 46 were confirmed. Out of these 46 samples only 22 samples were found antibiotic resistant.

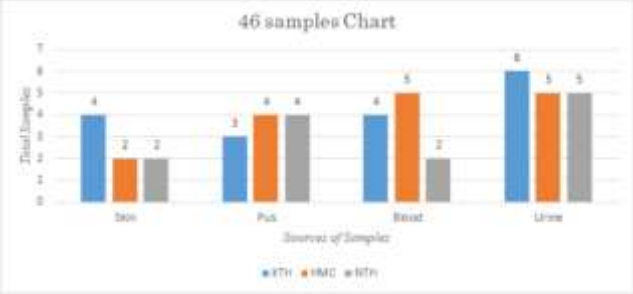

Figure 1. This figure shows the total number of samples which has shown growth on nutrient agar.

In skin samples almost all of the 8 species were found Gram positive. In pus samples, all 11 bacterial species were found Gram negative. In blood samples all 14 bacterial species were found Gram positive and in urine all of 16 bacterial species were found Gram negative. Catalase test showed all species were catalase positive. The species were found oxidase negative except one pus sample. Urease test turned out to be negative for all the species expect one bacterial specie. Disc sensitivity test results turned out that out of 46 bacterial species only 22 bacterial samples were antibiotic resistant.

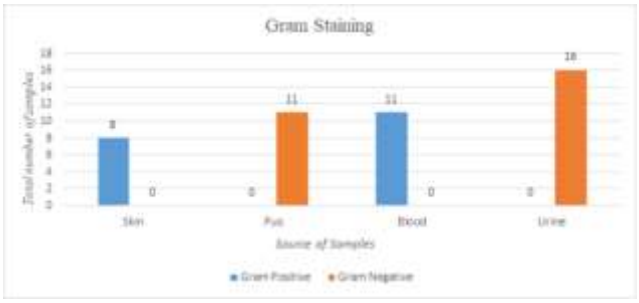

Figure 2. Gram Staining of the determining the bacterial samples.

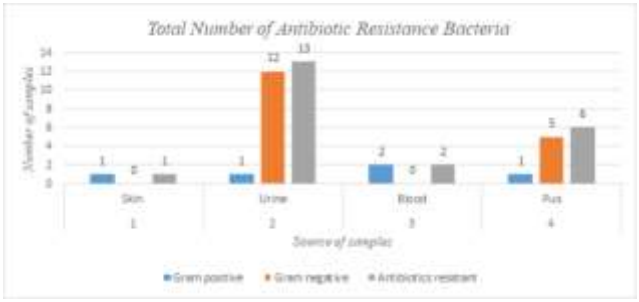

Figure 3. Total number of Antibiotic bacteria

Table-1. Antimicrobial tests for *S. aureus* isolated from blood, skin, pus and urine samples

Specie	amp <13	tzp <17	tec <17	cip <15	lev <13	dox <10	atm <17
Skin	9.46	12.07	13.08	12.26	12.49	8.22	14.25
Blood	7.27	12.94	13.34	13.38	11.33	8.11	15.11
Pus	7.11	12.18	13.17	14.23	12.54	8.55	13.35
Urine	7.76	11.88	12.40	12.52	12.43	8.36	14.38

Table 2. Antimicrobial tests for *P. aeruginosa* isolated from pus samples

Specie	amp <13	tzp <17	tec <17	cip <15	lev <13	dox <10	atm <17
Pus	7.21	12.35	13.39	13.31	12.27	8.27	13.33

Table 3. Antimicrobial tests for *K. pneumonia* isolated from urine samples

Specie	amp <13	tzp <17	tec <17	cip <15	lev <13	dox <10	atm <17
Urine	7.21	12.35	13.39	13.31	12.27	8.27	13.33

Table 4. Antimicrobial tests for *E. coli* isolated from blood and samples

Specie	amp <13	tzp <17	tec <17	cip <15	lev <13	dox <10	atm <17
Skin	7.56	12.55	13.34	13.38	12.45	8.25	13.39
Blood	7.53	12.44	13.42	13.35	12.39	8.37	13.66

Table 5. List of available Antibiotics used against bacterial sample.

S. No	Antibiotics	Symbols	Strength of antibiotic in µg	Zone of Inhibition		
				S	I	R
1	ampicillin	AMP	10	>17	14 - 17	<13
2	piperacillin-tazobactam	TZP	100 + 10	>21	18 - 20	<17
3	doxycycline	DOX	30	>14	11 - 13	<10
4	aztreonam	ATM	30	>21	18 - 20	<17
5	ciprofloxacin	CIP	5	>21	16 - 20	<15
6	levofloxacin	LEV	5	>17	14 - 16	<13
7	teicoplanin	TEC	30	>20	17 - 18	<17

DISCUSSION

After the discovery and frequent use of antibiotics the antibiotic resistance the rapidly increased [15, 16]. This rapid increase had a great impact of human population because of several reasons, like environmental stress, waste management and other inappropriate practices which lead this bacteria to antibiotic resistance [17, 18]. From past several years the antibiotic resistance increased rapidly which is due to the introduction of new antibiotic in the health care which not only lead to the bacterial resistance but also caused severe consequences to human health[19,20]. This is therefore related to the current study where researchers reports antibiotic resistance bacteria from different hospitals of Peshawar. This resistance was attributed to the frequent use of antibiotic by patient themselves and sometimes practitioners as well. Which lead the bacteria to become extreme drug resistance [21, 22].

After the frequent use of antimicrobial agents in many fields e.g. agriculture and veterinary, the bacterial population among these areas became resistant to all the applied antibiotics [23]. Many cases were reported showing a high bacterial resistance to antibiotics used in day care and health care centers. Some genes could be acquired from environment through animal feces through water and many other sources which ultimately reach to the humans and cause life threatening diseases [24]. As for the present research *Staphylococcal* strains from samples taken from hospitals were resistant [25].

CONCLUSION

In the collected samples, *Staphylococcus aureus* being Gram positive and present in skin, urine, pus and blood. Similarly *E.coli* as Gram-negative determine in all the tested samples i.e. skin, urine, pus and blood. *Pseudomonas aeruginosa* availability was in only pus and *Klebsiella pneumonia* in urine. All of these bacterial species were found resistant against available antibiotics. However, aztreonam antibiotic was determine being the highest effective antibiotic against *staphylococcus sp*, *E.coli*, *pseudomonas aeruginosa* and *Klebsiella pneumonia*.

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