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Antibiotic Susceptibility Profile of *Staphylococcus Aureus* and *Micrococcus Luteus* Isolated from Tap Water of Hayatabad Medical Complex and Cantonment General Hospital Peshawar

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Abstract

The development of antibiotic resistance in pathogenic bacteria has been a problem. Hospitals are where the probability of developing multidrug-resistant (MDR) bacteria is much higher. We investigated the presence of MDR bacteria in tap water collected from Hayatabad Medical Complex and cantonment board hospital, Peshawar. Bacterial strains were isolated from tap water, and their antibiotic sensitivity profile was investigated. Six commonly used antibiotics; Co-trimoxazole, gentamycin, clindamycin, augmentin, erythromycin, and ciprofloxacin. Two bacterial strains were isolated from the tap water and identified as *Staphylococcus aureus* and *Micrococcus luteus*. Both the bacterial strains were found resistant to clindamycin and augmentin antibiotics. *Micrococcus luteus* also showed resistance to clindamycin while sensitive to the rest of the antibiotics used during the experiment. The antibiotics gentamycin, erythromycin, and ciprofloxacin were highly effective against the isolated bacterial strains. Further investigation is suggested to find how these bacteria reach hospitals' sources and storage tanks. It is also indicated that tap water from other hospitals should be investigated for MDR bacteria.

Keywords: Multidrug resistance, bacteria, tap water, Hospital

Introduction

Staphylococcus aureus and *Micrococcus luteus* are gram-positive and nosocomial pathogenic bacteria that cause skin infections, meningitis, arthritis, bacteremia, pneumonia, food poisoning, and endocarditis by facilitating tissue attachment, invasion, and evading from host immune response. A methicillin-resistant *S. aureus* (MRSA) may increase the treatment cost, and high mortality and

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morbidity occur. However, new antibiotic discovery may also treat MRSA if not misused (Gnanamani, Hariharan, & Paul-Satyaseela, 2017). The researcher also reported that 17 previous cases of infective endocarditis are due to *Micrococcus* species (Khan, Aung, & Chaudhuri, 2019).

Antibiotic is a group of drugs that either kill or stop bacterial infection. In recent years the patient was first treated with antibiotics, due to which the antibiotic was misused and overused, and therefore bacteria become resistant and threat to public health (Spellberg & Gilbert, 2014). The microbial infection management was 1st documented in China and Greece. It was first discovered by Alexander, due to which it saved many lives because it treated serious bacterial infections in world war2 (Sengupta, Chattopadhyay, & Grossart, 2013). But soon after, those bacteria became resistant, and the need for new beta-lactam antibiotic drugs was discovered. In response to those, MDR bacteria were reported in America and soon after that pharmaceutical industry introduced some other new drugs to solve that problem(Sengupta et al., 2013; Spellberg & Gilbert, 2014). Antibiotic can save many patients live because it treats chronic infection like diabetes, end-stage renal disease, or rheumatoid arthritis (Gould & Bal, 2013). Moreover, it is also used in complicated surgery like organ transplants, joint replacements, or cardiac surgery (Gould & Bal, 2013; Wright, 2014).

The epidemiological studies show that resistance may be occurring by mutation and increased in many countries due to overuse of antibiotics available over the counter without prescription(Michael, Dominey-Howes, & Labbate, 2014). In bacteria, these resistance genes can be inherited by vertical and horizontal gene transfer(Read & Woods, 2014). The study reported that 30%-60% of antibiotics are incorrectly prescribed and used for ICUs, which increases resistance in bacteria (Lushniak, 2014; Luyt, Bréchot, Trouillet, & Chastre, 2014).In many countries, i.e. the U.S, 80% of antibiotics are used in livestock, which humans ingest to prevent infection that improves the animal's health and produce a higher quality of yields product (Golkar, Bagasra, & Pace, 2014). The antibiotic in these countries is sold for hygienic purposes in pesticides. It also affects environmental microbes and limits the development of immunity in children and adults (Golkar et al., 2014; Michael et al., 2014).

The molecular study shows that resistance properties of bacteria, i.e. normal flora, were first reported in farm areas to humans when they consumed meat products (Bartlett, Gilbert, & Spellberg, 2013). Due to economic crises in the society of pharmaceutical companies, a few antibiotics have been discovered (Bartlett et al., 2013; Piddock, 2012). Several new antibiotics have been cancelled due to the health regulatory authority (Gould & Bal, 2013; Piddock, 2012). Most of the antibiotic resistance bacteria (ARB) that are accelerated by antibiotic pressure are transferred from the hospital by the interactions of patients and Health Care Worker. Thus, their specific character and behaviour should be influenced. Many researchers reported that ARB might increase or decrease depending on the antibiotic class (Almagor et al., 2018). The antibiotic treatment may transmit the ARB when the patient misuse antibiotic due to which normal flora microbes eradicated and then ARB colonization occur. Thus 80% of colonized ARB-related patients are not treated with antibiotics(Pamer, 2016).The main aims and objectives of that study were to evaluate the antibiotic susceptibility profile of isolated bacterial strains from tap water in Hayatabad Medical Complex and Cantonment General Hospital.

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Material and methods

Sample collection

150 Tap water samples were collected from different wards of Hayatabad Medical Complex (HMC) and Cantonment General Hospital (CGH) Peshawar in sterilized glass bottles. The bottles were labelled properly, transported within an hour to the laboratory, and kept at 4℃ for analysis.

Serial dilution

Serial dilutions of each sample were made separately. Six test tubes that contained 9ml sterilized distilled water were taken. 1ml of the sample was mixed in the first test tube, then transferred 1ml of the dilution to the second test tube, then transferred 1ml dilution of the second test tube to the third test tube, and so on.

Isolation of *Staphylococcus aureus* and *Micrococcus luteus*

Nutrient agar and Mannitol Salt Agar (MSA) media isolated *S. aureus* and *Micrococcus luteus*. Each medium was prepared in an Erlenmeyer flask, sterilized in an autoclave at 121 ℃ for 15 mins, and then poured into Petri plates. After solidification, each dish was inoculated with 100 µl from10^{-2}, 10^{-4}, and 10^{-6} dilution. The spread plate method was used to spread the inoculums on each media plate using a sterilized glass spreader. The plates were then incubated for 24hours at 37℃ temperature for bacterial growth.

Pure Culture preparation

The next day colony was observed, and again Nutrient agar and MSA media were prepared and used to prepare pure culture. Each colony was then selected and streaked on nutrient agar and MSA plates. The plates were incubated at 37℃ for 24 hours to get pure culture.

Morphological and biochemical identification of *S. aureus* and *Micrococcus luteus*

The isolated bacteria, i.e. *S. aureus* and *Micrococcus luteus*, were examined by gram's staining test to differentiate between gram-positive and gram-negative bacteria and their morphology. Further identification of *S. aureus* and *Micrococcus luteus* was made by performing a series of biochemical tests using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology such as Coagulase, Catalase, TSI, Oxidase, Indole Mannitol, and Lactose fermentation test.

Antibiotic susceptibility determination

An antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method. Muller Hinton Agar has been accepted for international recognition for antimicrobial susceptibility testing. The different antibiotic discs at the final concentrations that are indicated were used, i.e. erythromycin, clindamycin, ciprofloxacin, gentamycin, augmentin, and cotrimoxazole. All these antibiotics were obtained from a local pharmacy store. The pure culture was picked from each sample, and each colony was transferred into 3 ml of sterile distilled water to prepare a bacterial suspension. Aliquots of 100µl from each suspension were spread-plated on Mueller-Hinton agar plates. Antibiotic discs were applied onto the plates using sterile needles, and the plates were incubated at 37°C for 24 hours. After incubation, the antibiotic inhibition zone diameters were measured, and the result was noted according to the Clinical and Laboratory Standards Institute (CLSI).

Result

Isolation of bacteria from hospital tap water

A total of 180 isolates were obtained from tap water samples collected from different wards of Hayatabad medical complex and Cantonment General Hospital Peshawar. Gram staining was done to observe the morphological character of all isolates. The gram staining result shows that all the isolated bacteria were gram-positive, as given in Table 1 and Fig 1. Table 1 presents the percentage presence of isolated bacterial strains.

Table 1. Show gram staining and occurrence of bacterial culture in the water sample.

S. No	Bacterial isolates	Gram reaction	No. of isolates	Percentages
1	*Staphylococcus aureus*	Positive	150	83.33%
2	*Micrococcus luteus*	Positive	30	16.99%
Total			**180**	**100%**

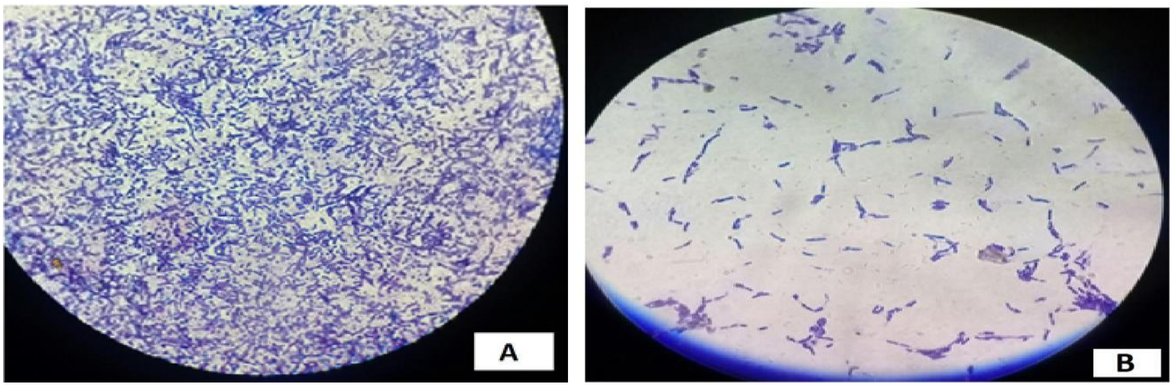

Figure 1. Show gram staining of gram-positive bacteria (A) *Staphylococcus aureus* and (B) *Micrococcus luteus.*

Identification of bacterial species through the biochemical test

These isolates were identified as *Staphylococcus aureus* and *Micrococcus luteus* through biochemical tests (Catalase test, Oxidase test, Indole, Coagulase test, TSI, Mannitol test, and Lactose fermentation test), morphology, and gram staining, as shown in Table 2. Figures 3-6 show the results of various biochemical tests.

Table 2. Biochemical and morphological characterization of isolated strains.

S. No.	Biochemical test	*Staphylococcus aureus*	*Micrococcus luteus*
1	Catalase	Positive (+)	Positive (+)
2	Coagulase	Positive (+)	Negative (-)
3	Mannitol	Negative (-)	Positive (+)
4	Lactose test	Negative (-)	Negative (-)
5	Oxidase	Negative (-)	Positive (+)
6	Indole	Negative (-)	Negative (-)
7	TSI	A/A	A/K

Key: A/A: (acidic slant/acidic butt), A/K: (acidic butt/alkaline slant), TSI: (Triple Sugar Iron).

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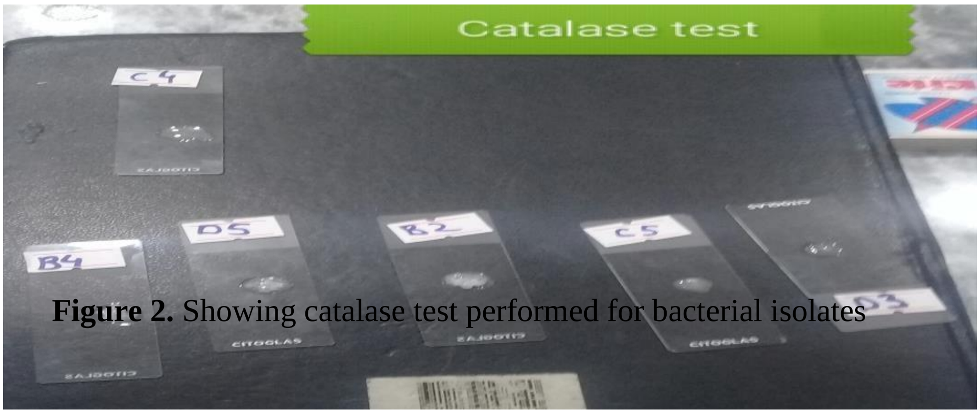

Figure 2. Showing catalase test performed for bacterial isolates

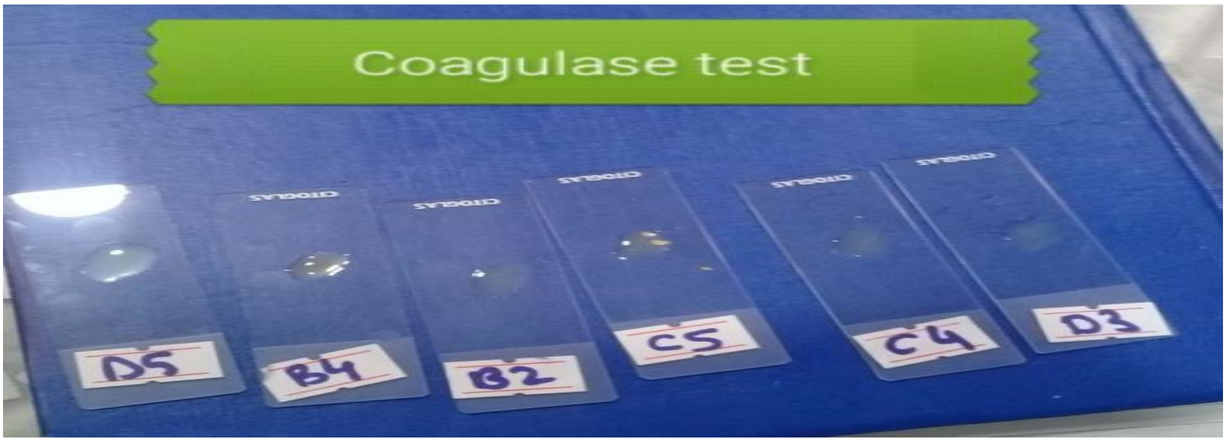

Figure 4. Showing mannitol test for bacterial isolates

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Antibiotic susceptibility profile

Table 3 and fig. 7 and 8 show the antibiotic susceptibility patterns of each isolate. *Staphylococcus aureus* was highly susceptible to Gentamycin, Erythromycin, and Ciprofloxacin and resistant to Clindamycin and Augmentin antibiotics while showing intermediate sensitivity to Co-trimoxazole. Moreover, *Micrococcus luteus* was highly sensitive to Gentamycin, Erythromycin, and Ciprofloxacin while resistant to Co-trimoxazole, Clindamycin and Augmentinantibiotic.

Table 3. Antibiotic susceptibility profiles of bacterial isolates

S.No.	Antibiotic	Mean diameter of inhibition zone	
		Staphylococcus aureus	*Micrococcus luteus*
1	Cotrimoxazole	18 mm	8 mm
2	Gentamycin	30mm	25mm
3	Clindamycin	5 mm	6 mm
4	Augmentin	1 mm	5mm
5	Erythromycin	28 mm	28mm
6	Ciprofloxacin	28 mm	28mm
7	Ceftriaxone	18 mm	15 mm

Note: Zoneofinhibition:0.13mm=resistance,14-17mm=intermediate,18mm and above =sensitivity.

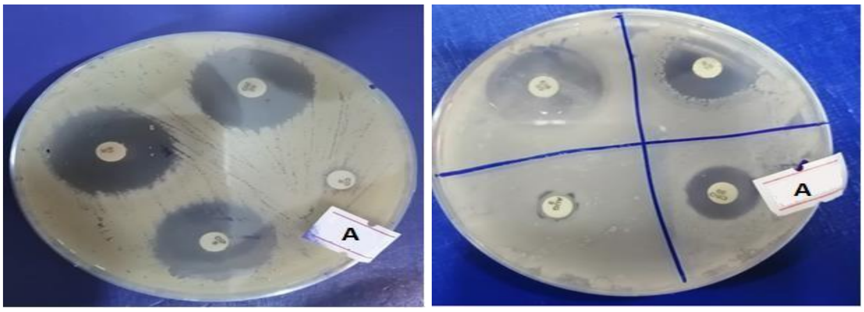

Figure 6.Antibiotic susceptibility profile of *Staphylococcus aureus*

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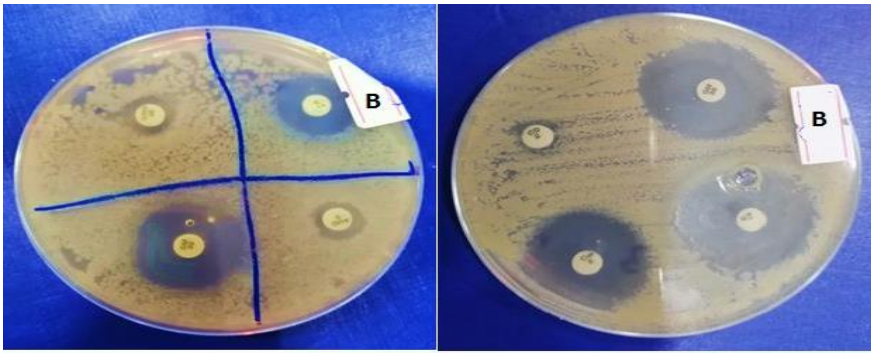

Figure 7. Antibiotic susceptibility profile of *Micrococcus luteus*

Discussions

The emergence of resistance in pathogenic bacteria against antibiotics has become a major public health concern. The extensive use of antibiotics in hospitals is considered one of the causes of spreading resistance in pathogenic bacteria (Xi et al., 2009). Several investigators have isolated antibiotic-resistant bacterial strains from solid and liquid waste hospitals (Adnan, Sultana, Islam, Nandi, & Hossain, 2013). There are chances that pathogenic bacteria could get entry into the hospital drinking water sources and storage tanks. We have investigated the presence of multidrug-resistant pathogenic bacterial strains in tap water collected from HMC and CGH in Peshawar. We have isolated two pathogenic bacterial strains, i.e. *Staphylococcus aureus* and *Micrococcus luteus*, which showed resistance to different antibiotics used during the experiments. Both the isolated species are pathogenic and have produced several infections in humans(Gnanamani et al., 2017). Previously, these bacteria have been isolated from hospital wastewater (treated and non-treated), but we have separated them from hospital tap water in the current study.

Kalaiselvi*et al*. (2016) isolated *Staphylococcus aureus* strains from hospital-generated recycled water and checked their antibiotic resistance profile. They found that the isolated *S. aureus* was resistant to several antibiotics, including gentamycin, erythromycin, and ciprofloxacin (Kalaiselvi, Mangayarkarasi, Balakrishnan, & Chitraleka, 2016). Our results also show that the isolated *Staphylococcus aureus* was resistant to ampicillin and cotrimoxazole while susceptible to gentamycin, clindamycin, Ofloxacin and Ciprofloxacin. In another study, Thompson *et al*. (2013) isolated *S. aureus* from the hospital environment, resistant to ciprofloxacin and gentamycin. The bacterial *Micrococcus luteus* were found resistant to Augmentin, Co-trimoxazole and Clindamycin. The implication of *S. aureus* in toxic shock syndrome generates immediate concern about the possibility that drinking water may be a source of this syndrome (Thompson, Gündoğdu, Stratton, & Katouli, 2013).

The presence of antibiotic-resistant bacteria in tap water may be due to inadequate/ unhygienic sanitation systems. The prevalence of antibiotic-resistant bacteria poses a great challenge to clinicians, and the consumption of water containing these antibiotic microbes may prolong the treatment of water-borne diseases. This implies that treating water-borne diseases with these antibiotics may be inappropriate and require new and most expensive antibiotics. This study recommended that stringent measures be taken to prevent their occurrence.

Conclusion

Emergences of resistance in bacteria against antibiotics have compromised the effectiveness of antibiotics used against these bacteria. From this study, it has been concluded that tap water was collected from Hayatabad medical complex and cantonment board hospital Peshawar contain MDR bacterial strains and is not safe for drinking and washing purposes.The bacterial strains *S. aureus* and *M. luteus*are pathogenic, and their presence in hospital tap water is a serious public health concern.

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