Short Research Article

Isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA) from mobile phone of University students.

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Abstract

Mobile phone is a wireless handheld electronic device that allow users to make and receive calls. They are becoming fomites for the transmission of methicillin resistant *S. aureus* (MRSA), due to unhealthy handling of these devices by users who do not have regard for hygiene, thereby making the gadget prone to pathogenic microorganisms. This study was carried out to isolate Methicillin-resistant *Staphylococcus aureus* (MRSA) from mobile phone of University students and to propose possible preventive measures against the organism. A total of 60 mobile phones were randomly selected from University students and swab from surfaces of these mobile phones were collected using disposable sterile cotton swabs moistened with sterile normal saline. These samples were inoculated onto plates containing Mannitol salt agar and were incubated aerobically at 37^0C for 24 hours. Out of the sixty (60) mobile phone samples that were analyzed, 41 (68.3%) of the samples were contaminated with Methicillin-resistant *Staphylococcus aureus.* This was determined using Oxacillin (Oxoid) 10µg sensitivity disc which 41 (68.3%) of the samples were sensitive to.This study showed that mobile phones are carriers of MRSA and cleanliness or good hygiene practices such as constant hand washing, regular disinfection of mobile phones with 70% alcohol and avoiding the use of mobile phones in the toilet are necessary measures that will help in reducing the occurrence of this organism. These measures can make mobile phones MRSA-free thereby reducing morbidity, mortality and the cost of medical treatment.

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The study showed the mobile phones are carriers of MRSA. The second aspect is a form of recommendation. So, do rephrase/reconstruct the sentence.

Keywords: Mobile phone, Fomite, MRSA, Antibiotic resistance, Poor hygiene, Infection control

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Introduction

Mobile phone is a wireless handheld electronic device that allow users to make and receive calls. Today, cell phones come up with a range of applications such as internet browsing, e-mail, game sites, social networking sites, music and video apps, radio, E-books, dictionary, etc [4,12]. The use of mobile phone has expanded rapidly on a global scale and has brought numerous changes in the daily lifestyle of individuals [24]. It has become part and parcel of modern life to the extent that to live a life without mobile phone is an impossible task for most people [16].

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Because of the achievements and benefits associated with it, it is easy to overlook its health hazard; this is against the background that many users may have no regard for personal hygiene, and the number of people who may use the same phone [16,19,12]. However, unhygienic ways of handling these gadgets make them a leading reservoir of array of pathogenic microorganisms [24,20,2,4,12] especially those associated with the skin resulting in the spread of different microorganisms from user to user [2]. Mobile phones might act as fomites to transmit various microorganisms since they are always used in contaminated areas such as toilet which are loaded with microorganisms [20]. Contamination from the skin, anal region, wounds, nasal secretions and aerosols generated by sneezing and coughing are potential sources of transferring microorganisms to mobile phones during handling. Due to their personal nature and proximity to sensitive part of our bodies like faces, ears, lips, and hands of users, mobile phones could become absolute reservoirs of pathogens that might cause infections. [12,20,19,11]. This can also be a source of infection when handled by another user. The moisture and ideal temperature of the human body, particularly the palms of the hands and the heat generated by these gadgets could contribute to the growth of these pathogenic

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organisms on these devices. Constant use of these devices and lack of disinfection can make them possible routes for transmission of bacterial pathogens, including multi-drug-resistant organisms [5,22].

Staphyloccus aureus is a global human pathogen and a common cause of invasive and life threatening infections. It is the most common cause of folliculitis, boils, furncles, carbuncles, community associated cellulitis, endocarditis and a common cause of bacteremia. Also, it can cause post-operative wound infections, food poisoning, pneumonia in infants, debilitated and immunocompromised individuals [13]. *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase- resistant β – lactams, the most commonly used class of antibiotics for skin infection. These strains were termed methicillin resistant *S. aureus* or MRSA, a term that implies cross-resistance to all β – lactams including all penicillins and cephalosporins [13,1,17]. This drug resistance has developed rapidly and continues to evolve with each new medication developed to combat this infectious agent [7].

Methicillin is an antibiotic that was formally used in the treatment of bacterial infection cause by microorganisms of the genus *Staphylococcus aureus*. Methicillin contains modification of the original penicillin structure. Most strain of *Staphylococcus aureus* produce enzyme penicillinase (betalactamase) which acts by hydrolyzing the beta-lactam ring which is the central antimicrobial activity of the antibiotic. Methicillin resistance result from the production of an alternative penicillin binding protein PBP2A or PBP2, encoding by the Mac gene on the Staphylococcus cassette chromosome me (SCC me) which is a mobile genetic element supposedly acquired through horizontal gene transfer from Cons. MRSA strains have an altered protein penicillin binding protein (PBP2) that shows low affinity for all beta- lactam antibiotics (Penicillin and

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Cephalosporin's) [9]. The lack of binding of β-lactams to Penicillin-Binding proteins (PBPs) is the main cause of *Staphylococcus aureus* resistance to the antibiotics [14].

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a global health concern over the years [23,14]. It is a substantial public health problem worldwide, causing significant morbidity and mortality [7,17,10] and elevated health care costs. An example of the increased morbidity and mortality associated with MRSA can be seen when comparing the yearly infection rates and mortality rates in the United States for MRSA, AIDS, viral hepatitis, and tuberculosis. Methicillin-resistant *S. aureus* is estimated to cause more infections than the other diseases combined and more deaths per year than AIDS [7]. The aim of this study was to isolate Methicillin-resistant *Staphylococcus aureus* (MRSA) from mobile phone of University students and to propose possible preventive measures against the organism.

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Materials and methods

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Your sample size is small relative to an average expected population of students in any university community.
How would a sample size of 60 represent a student population in a university (which is usually at least 10,000).
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A. Sample Collection

A total of 60 mobile phones were randomly selected from University students. Swabs from surfaces of the mobile phones were collected using disposable sterile cotton swabs moistened with sterile normal saline.

B. Isolation of Bacteria

Samples from the mobile phones were cultured onto plates containing Mannitol salt agar. The plates were then inverted and incubated aerobically at 37^0C for 24 hours. Pure cultures were obtained by sub-culturing distinct colonies onto nutrient agar plates which were also incubated aerobically at 37^0C for 24 hours.

C. Identification of Bacterial Isolates

The bacterial isolates were identified according to the standard microbiological methods described by [8]. Identification was done using Gram's staining and appropriate biochemical tests. They include Indole, Oxidase, Citrate utilization, catalase, coagulase and sugar fermentation tests.

Gram staining: A smear of each bacteria isolate was made on different clean grease free slides with a sterile wire loop and left to dry and after they were heat fixed and allowed to cool. Then the different smears were stained with crystal violet for 30-60 seconds and rapidly washed off with clean water. Then the smears were stained with lugol's iodine for 30-60 seconds and rapidly washed off with clean water. The smears were decolourized with 75% alcohol for 30 seconds and washed out immediately with clean water. Then the smears were stained with safaranine for 30-60 seconds and washed off immediately with clean water. The stained slides were then allowed to air dry. After drying, a few drops of oil immersion were dropped on the stained smears and viewed under microscope (100 oil objective lens) to check for the microscopic properties of the organism. The gram negative cells appear red pink in colour while gram positive cells appear purple or blue.

Catalase test: Two mls (2mls) of hydrogen peroxide solution was poured into a clean test tube and using a wire loop, a good growth of the test organism was removed and immersed into the hydrogen peroxide solution, active bubbling indicated a positive result while no release of bubble indicated a negative test.

Coagulase test: A drop of physiological saline was placed on a clean slide and a loopful of the isolate was emulsified into it then a loopful of plasma was placed on it. It was rocked and clumping indicated a positive result while no clumping indicated negative result.

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Indole test: The little portion of each of the isolate was inoculated into 5mls of sterile peptone water which was added in different test tubes using wire loop and then the test tubes containing the organisms were left to incubate at 37°C for 48 hours. After incubation, 3-4 drops of indole reagent were added and it was mixed gently. A red surface layer after 10 minutes gave a positive result while no red surface layer after 10 minutes gave a negative result.

Citrate utilization test: The test organisms were inoculated into Simmon citrate agar slant and incubated for 24 hours at room temperature. The appearance of growth with blue colour indicated positive result while green colour indicated negative result.

Urease test: A 24 hours culture of each of the isolates was streaked into the surface of urea agar slant medium contained in bijou bottle; they were incubated at 27°C for 24 hours. Purple pink colour indicated positive test.

Sugar fermentation: The ability of an organism to ferment various sugars or digest carbohydrate is indicated by the production of acid and gas. The test organism was incubated in peptone water both containing 1% solution of desired sugar. Phenol red was added as an indicator. Then an inverted durham tube was inserted in the culture tube and was incubated at 37°C for 24 hours. Acid production was indicated by the change of colour of the medium to yellow. If gas is produced, it collects in durham tubes, which rise up the culture tubes.

D. Antibiotic Susceptibility Test

Kirby–Bauer disc diffusion susceptibility test method was used to determine the sensitivity or resistance of the organism to antibiotics. The pure culture of the test organisms were adjusted to 0.5 MacFaland turbidity standard and were inoculated on different plates containing Muller-hinton agar. They were spread using sterile spreader after which sterile forceps was used to place the

various antibiotic discs aseptically on the plates. The plates were incubated at 37^0C for 24hours and the diameter of zone of inhibition was measured, recorded and interpreted according to the guidelines of clinical laboratory standard institute (CLSI, 2009).

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E. Isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* were determined using Oxacillin (Oxoid) 10µg sensitivity disc. The pure culture of the test organisms that were resistant to the test antibiotics were adjusted to 0.5 MacFaland turbidity standard and were inoculated on different plates containing Muller-hinton agar. They were spread using sterile spreader after which sterile forceps was used to place the oxacillin disc aseptically on the plates. The plates were incubated at 37^0C for 24hours and the diameter of zone of inhibition was measured, recorded and interpreted according to the guidelines of clinical laboratory standard institute (CLSI, 2009).

Results and Discussion

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The isolates were identified based on their cultural, morphological and biochemical characteristics (table 1).

Out of the sixty (60) mobile phone samples that were analyzed, 41 (68.3%) of the samples were contaminated with Methicillin-resistant *Staphylococcus aureus* while 19 (31.7%) were not contaminated (table 2).

Also, the antibiotic sensitivity pattern of the isolates were determined. It was observed that all the MRSA isolates were resistant to all the test antibiotics that were used for the analysis except Oxacillin which all the 41 (fourty-one) samples were sensitive to. This confirmed that the isolated organisms were MRSA (table 3).

Table 1: Result of biochemical characteristics of the isolated organism

Biochemical test	**Result**
Gram reaction	Gram positive cocci in clusters
Catalase	+ve
Oxidase	-ve
Coagulase	+ve
Citrate	-ve
Indole	-ve
Urease	+ve
Sugar fermentation	
Glucose	+ve
Fructose	+ve
Maltose	+ve
Mannose	+ve
Lactose	+ve

Table 2: Prevalence of MRSA isolates on the samples

Organism isolated	**Number of samples**	**Number of positive samples (%)**	**Number of negative samples (%)**
MRSA	60	41 (68.3%)	19 (31.7)

Table 3: Result of Antibiotic Susceptibility pattern of MRSA

Antibiotics	**Number of isolates**	**Number sensitive**	**Number resistant**	**Disc potency (µg)**	**Zone of Inhibition (Mean)(mm)**
Amoxicillin	41	0	41	20	0
Chloamphenicol	41	0	41	30	8
Erythromycin	41	0	41	30	3
Ciprofloxacin	41	0	41	10	0
Rifampicin	41	0	41	20	3
Gentamycin	41	0	41	10	9
Streptomycin	41	0	41	30	2
Ampicillin	41	0	41	20	0
Septrin	41	0	41	30	2
Oxacillin	41	41	0	10	25

Mobile phones have become one of the most important electronic device that is used in our society. Although they are handy, they may be hazardous to human health due to the presence of harmful organisms been harbored on their surfaces. Therefore, mobile phones might act as fomites for the transmission of pathogenic microorganisms when handled unhygienically. Contamination from the skin, anal region, wounds, nasal secretions and aerosols generated by sneezing and coughing are potential sources of transferring microorganisms to mobile phones during handling [12]. They may harbor different pathogenic bacteria because they are commonly handled unhygienically regardless of poor sanitary condition of the hands that are not always disinfected. The use of such mobile phone serves as a potential vehicle for the spread of pathogenic pathogens including multidrug-resistant pathogens such as MRSA [19].

The result obtained from this study shows that out of 60 (sixty) mobile phones that were analyzed, 41 (68.3%) were contaminated with MRSA. It has been observed that constant handling of contaminated mobile phone exposes the user to MRSA which can be detrimental to health. These devices can also be contaminated when used in toilets and bathrooms and when handled with dirty/ contaminated hands without disinfecting them. These can predispose the user to pathogenic organisms which is harmful to health. These microorganisms can always be transmitted from one user to another when adequate hygienic measures are not maintained. Therefore, sharing mobile phones among individuals may directly facilitate the spread of these pathogenic bacteria [18]. Individuals with MRSA colonization have an increased risk of subsequent infection and are important source of person – person transmission [6,3]. Previous reports have revealed that mobile phones may be contaminated with pathogenic bacteria including MRSA Methicillin-resistant *Staphylococcus aureus* when not handled hygienically [15].

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Therefore, maintaining good hand and body hygiene, regular disinfection of mobile phones and avoiding the use of mobile phones in the toilet will help in reducing or preventing the occurrence of this organism.

The result of this study agrees with the work of [19] who isolated thirty (26.8%) bacterial isolates which were detected as Methicillin Resistant *S. aureus* (MRSA) from mobile phones and [9], who also isolated 45.46% of MRSA associated with mobile phones. It is also in conformity with the work of [21,20,15] who isolated MRSA from mobile phones.

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MRSA is a highly pathogenic organism of public health concern due to its resistance to β- lactam antibiotics. It can lead to skin and soft tissue infection, acute bacterial endocarditis, bone and joint infection etc. It is responsible for a range of infections, from skin and wound infection to pneumonia and bloodstream infections [23,2]. It is a common bacterial infection that is a significant source of illness and mortality globally [6].

The ideal treatment for MRSA remain challenging, and the quest for new antibiotic and advanced drug delivery systems with safety profile is necessary to ensure treating MRSA infections adequately in the future [6]. The result of this study confirms that MRSA was quite resistant to the test antibiotics as shown on table 3. The organism is highly resistant to methicillin and also to other β- lactam antibiotics and this has a great implication in the treatment of the infection caused by MRSA.

Even with the ongoing development of new antibiotics, active surveillance efforts and advances in infection prevention, MRSA remains a prominent pathogen with persistently high mortality and morbidity[17].

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Conclusion

This study showed that mobile phones are carriers of MRSA and cleanliness or good hygienic practices are necessary in reducing the occurrence of this organism. Simple control measures are also very important in reducing the potential contamination of the device. This can be achieved through constant hand washing, regular disinfection of mobile phones with 70% alcohol and avoiding the use of mobile phones in the toilet. Some of these measures can make their surfaces MRSA-free thereby reducing morbidity and mortality and the cost of treatment.

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