ANTIBIOTIC SUSCIPTIBILITY TESTING OF BACTERIA ISOLATED FROM MOBILE PHONES

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Abstract

Introduction: Now a days mobile phones are found everywhere. They have become a necessity. This study aims at finding the presence or absence of drug resistant bacteria on mobile phones.

Method: A total of 5 samples were collected from the cell phones of the students of biotechnology department, Modern college, Shivajinagar. In the lab, swabs were taken from the phones and were streaked on nutrient agar and MacConkey agar. The subsequent bacterial isolates were identified by their cultural, morphological and biochemical characteristics.

Result: The mean of bacterial count per mL was found to be $1.009*10^9$ cfu/ml. Bacteria encountered mostly consisted of gram negative (80%) microorganisms. Isolates belonging to sample A were the most resistant of all and showed resistance to multiple antibiotics used in this study. We have found that the most effective antibiotics against the isolated bacteria were ciprofloxacin and imipenem. Even though the sample A, C, D, E were gram negative in nature they shows resistivity to aztreonam and ceftazidime. Analysis of the MAR index of isolates showed that all 5 isolated bacteria had ratios above 0.2, indicating high resistance.

Conclusion: Pathogenic bacteria were detected with multiple antibiotic resistance indexes. Hands and mobile phones can act as carriers for infectious agents, suggesting the need for hand hygiene and disinfecting mobile phones surfaces.

Keywords: Bacteria, Antibiotic, Antibiotic Resistance, Student, Mobile Phone.

1. Introduction

Hand hygiene is considered the most important habit to prevent infections and the spread of microorganism's pathogens. The common people often believe that microbes are only present in rubbish and dumps, in research labs, in sick people, in hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where germs occur and how are they transmitted could be the cause of health problems. Microorganisms are found almost everywhere in air, water, soil, food, plants and animals, including humans and may be transmitted, either directly, through hand-to hand contact, or indirectly via food or other inanimate objects such as cell phones, money and coins (Martina et.al. 2019).

Now a days cell phones have become one of the most vital accessories of professional and social life. A mobile or cellular phone is a long-range, portable electronic device for personal communication in less than two decades, mobile phones have gone from being uncommon, costly pieces of equipment used mainly by the business elite, to common, low-cost personal items. In many countries, mobile phones outnumber landline telephones, as many adults and children now own their own personal mobile phones (Mushabati et.al. 2021).

The constant handling of phone by different users exposes to an array of microorganisms and makes it a good carrier of for microbes, especially those associated with the skin resulting in the spread of different microorganisms from user to user. Therefore, mobile phone is not only transmitting messages but also microorganisms and many of which are reported to be pathogenic. Maximum human pathogenic bacteria are mesophilic, referring to maximum colony growth at temperature ranging from 20°C to 45°C (37°C most favorable). On this note, studies include that the average temperature of the smart phone that we use varies between 25°C and 32°C in normal condition. During charge intake, the phone temperature might reach to 37°C—43°C, making the phone a favorable breeding zone for the bacteria. Since females are more comfortable carrying the mobile phones in their handbags, a study reports that the heat generated by the mobile phones and the internal areas of the bag provides support to breeding of bacteria. (Rozario et.al.2019).

Several studies have also shown the presence of bacteria on the surface of the mobile phone, especially in medical facilities. A study reported that many species of commonly found bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Neisseria sicca, Micrococcus luteus, Proteus mirabilis, Bacillus subtilis,* and *Enterobacter aerogenes* were identified on mobile phone surface. Other studies included that coagulase-negative *staphylococcus, S. aureus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Bacillus spp.,* and *P. aeruginosa* were also present on the surface of the mobile phone. (Martina et.al.2019; Taiwo et.al.2021; Rozario et.al.2029; Waleed et.al.2019; Mushabati et.al.2021; Sadiq et.al.2020)

The emergence of antimicrobial resistance is an important issue associated with nosocomial infections and most nosocomial infections are often caused by antibiotic resistant organism. Antibiotic resistance increases the morbidity and mortality associated with infections and contributes substantially to rising costs of care resulting from prolonged hospital and the need for expensive drugs. (Gashaw et.al. 2014)

The current study aims to detect potential pathogenic and multidrug-resistant (MDR) bacteria on the surface of mobile phones used by students.

2. Material and methods

2.1 Study Area and Design: This study was conducted in Modern College Shivaji Nagar Pune. Mobile phones of 5 students were involved in the current study. Before the collection of the swab samples from the surface of the Mobile phones, users completed a self-administered questionnaire form consisting of basic questions about the frequency of daily use of their phone, how often they wash their hands and clean their mobile phones (Sadiq T. et.al.2020).

2.2 Sample Collection: A total of 5 swab samples were collected from the touch screen surfaces of mobile phones which belong to 5 students at modern College Shivaji Nagar, faculty of biotechnology. This age group was preferred as they use mobile phones frequently in their daily and social lives. Sterile cotton swabs in saline were used to collect samples. (Sadiq T. et.al.2020). The swabs were then transferred into peptone water (5%).

2.3 Laboratory Methods and Procedures: Laboratory analysis was undertaken in the laboratories of the Department of biotechnology, Modern college, Shivaji Nagar, Pune.

Inoculation: The cotton end was cut off and soaked in 10ml sterile peptone water and incubated aerobically over-night at 37°C. (Tagoe et.al. 2011). After incubation, the samples were spread on nutrient agar plate and MacConkey agar plate.

2.4 Quantification of Bacteria: Serial dilutions from the resulting growth from the peptone water medium were spread plated on nutrient agar (NA) and incubated for 24hrs at 37oC under aerobic condition. The number of estimated Colony Forming Units (CFU) for each sample was then counted (Tagoe D N, et.al. 2011).

2.5 Identification of Organisms: Five colonies were randomly chosen and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase, and Urease test (IMViC test) (Tagoe et.al. 2011) and their gram nature was also identified. They were identified using Gram's staining, colony morphology and appropriate biochemical tests (Selim, et.al.2011; Tagoe, et.al. 2011).

2.6 Antibiotic Susceptibility Test (AST): Antibiotic susceptibility was determined by the agar diffusion technique on Mueller-Hinton agar (Kirby-BauerBiote NCCLS modified disc diffusion technique) using 10 antibiotic discs (Biotech Lab. UK) corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria such as Ciprofloxacin (CIP) 5mcg, Imipenem (IPM) 10mcg, Aztreonam (AT) 30mcg, Ceftazidime (CAZ) 30mcg, Piperacillin/Tazobactum (PIT) 100/10mcg, cefoperazone/sulbactum (CFS) 75/10mcg, Cefotaxime/clavulonic acid 30/10mcg, Piperacillin 100mcg, Cefetaxime 30mcg, Ampicillin 10mcg (Selim et.al. 2015; Momani et.al. 2019; Tagoe et.al.2011;Sadiq et.al.2020)

2.7 Multiple antibiotic resistance Index: Multiple antibiotic resistance index was determined to investigate the level of resistance among the isolated bacteria and was calculated according to the following equation:

$$Multipls\ Resistance\ Index = \frac{No.of\ antimicrobials\ to\ which\ the\ isolate\ is\ resistant}{No.of\ antimicrobials\ to\ which\ the\ isolate\ is\ subjected}$$

(Waleed et.al.2019).

3. Results and Discussion

General characteristics of the study participants and their response to the questions related to the use of mobile phones were noted (Table no. 1). A total of 73 responses were acquired in this study. The responses given by the participants regarding the use of mobile phones are summarized in table 1. Out of these 73 responses, five students of biotechnology department were chosen to participate in this study. The mean age of the participants was 21 years. Participants have been using their phone for approximately 2 years belonging to phone companies like Samsung, vivo, Xiaomi, Apple, Oppo, etc. companies. (Bodena et.al. 2019).

Table 1: Participants response for questions related to use of mobile phones

Questions	**Responses**	
What is your age?	31 (15-20 years)	42 (21-40 years)
What is your gender?	male (26)	female (47)
Do you consume antibiotics?	Yes (44)	No (29)
Do you discard remaining leftover medication	Yes (52)	No (21)
Do you consult doctor before starting an antibiotic?	Yes (50)	No (23)
Do you check expiry date of antibiotic before using it?	Yes (68)	No (5)
Do you know there are many micro-organisms on your phone?	Yes (61)	No (12)
Do you wash your hands before touching phone?	Yes (16)	No (57)
Do you know that *E.coli*, *S.aureus*, *B.subtilis*, etc. bacteria are found on phone?	Yes (46)	No (27)

3.1 Quantification of bacteria:

After taking the swabs off the phones, it was found that all 5 mobile phones were contaminated with microorganisms. After looking at the counts, it was found that all phones used in the study had around 10^8 to 10^9 cfu/ml bacteria (Table 2). A study done by Kõljalg et al. found ten times higher number of bacteria than that found in a similar study among university students in Germany where the average bacterial load on phones was 1.37 cfu/cm^2

Table 2: Number microorganisms found on mobile phone screens

Sample no.	cfu/ml
1	$2.80*10^9$
2	$9*10^8$
3	$5.65*10^8$
4	$1.3*10^8$
5	$6.5*10^8$

3.2 Isolation and Identification of Bacteria:

On swabbing the phone samples on different medias, various types of microorganisms were seen (Table 3). Eighteen different colonies and their colony characteristics were noted, out of these five random colonies were selected for further experimentation. It can be seen that they all have different morphological characteristics which means that they might as well have different biochemical characteristics (Table 4). After performing gram staining, it was found that about 80% microorganisms were gram negative in nature. In order to identify the genus and species further investigations need to be done.

Table no. 3 Colony characteristics

Colony No.	Size	Shape	Color	Opacity	Consistency	Elevation	Margin
1	Small	Irregular	Whitish	Opaque	Buttery	Raised	Undulate
2	Small	Circular	Pink On Macconkey	Opaque	Dry	Convex	Entire
3	Large	Irregular	Creamy Yellow	Opaque	Dry	Flat	Undulate
4	Small	Circular	Creamy Yellow	Opaque	Dry	Raised	Entire
5	Large	Punctiform	White	Transluecent	Dry	Umbilicate	Lobate
6	Large	Irregular	Creamy Yellow	Opaque	Buttery	Convex	Entire
7	Small	Circular	Creamy Yellow	Opaque	Buttery	Convex	Entire
8	Large	Punctiform	Pink On Macconkey	Transluecent	Dry	Umbilicate	Entire
9	Large	Punctiform	White On Macconkey	Transluecent	Dry	Umbilicate	Entire
10	Large	Irregular	Creamy Yellow	Opaque	Buttery	Umbilicate	Curled
11	Large	Irregular	Creamy Yellow	Opaque	Buttery	Raised	Undulate
12	Small	Circular	Pink On Macconkey	Opaque	Dry	Dome Shaped	Entire
13	Small	Circular	Whitish Yellow	Opaque	Dry	Flat	Entire
14	Large	Circular	Whitish Yellow	Transluecent	Buttery	Raised	Entire

15	Large	Punctiform	Whitish Yellow	Opaque	Buttery	Umbilicate	Lobate
16	Small	Punctiform	Pink On Macconkey	Opaque	Buttery	Dome Shaped	Entire
17	Large	Rhizoid	Whitish Yellow	Opaque	Buttery	Raised	Filamentous
18	Large	Irregular	Whitish Yellow	Opaque	Buttery	Raised	Lobate

Table 4: Biochemical characteristics

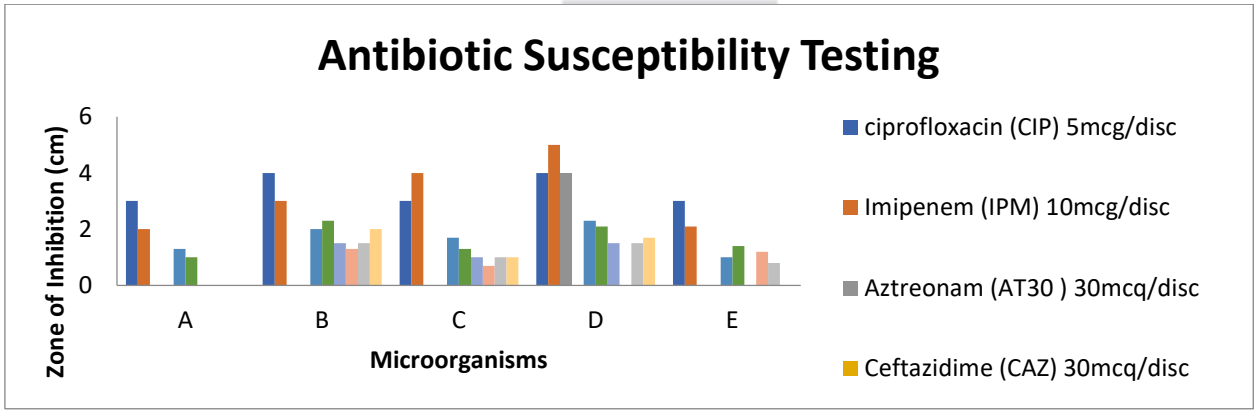
	Samples				
Tests	**A**	**B**	**C**	**D**	**E**
Indole Test	Negative	Negative	Positive	Negative	Positive
Methyl Red	Positive	Positive	Positive	Positive	Positive
VP Test	Positive	Positive	Positive	Negative	Negative
Lactose Test	Positive	Positive	Positive	Positive	Positive
Citrate Utilization Test	Positive	Positive	Positive	Negative	Positive
Oxidase Test	Positive	Positive	Positive	Negative	Positive
Catalase Test	Negative	Positive	Positive	Positive	Negative
Gram Nature	Negative	Positive	Negative	Negative	Negative

3.3 Antimicrobial susceptibility testing:

The antibiotic profile of the isolates showed a big variation shown . Although some bacterial species were susceptible to all antibiotics, others were resistant to as many as 8 antibiotics. The results of antibiotic susceptibility testing are shown in graph 1. It can be seen that microorganism designated as A is the most resistant of all. A as well as E were found to be sensitive to only ciprofloxacin and imipenem. On the other hand B is sensitive to all the antibiotics except aztreonam and ceftazidime. C was found to be sensitive to ciprofloxacin, imipenem and piperacillin. D was resistant to aztreonam, ceftazidime and piperacillin. On further deductions, it can be said that all the microorganisms were resistant to aztrionam and ceftazimide and were sensitive to ciprofloxacin and imipenem.

In recent studies by Bodena, it was found that ceftriaxone (80.6%), ciprofloxacin (77.3%), and gentamicin (72.7%) showed higher activity against bacterial isolates, while ampicillin and trimethoprim-sulfamethoxazole had less effect with a resistance rate of 61.6% and 56.9%, respectively. There was no significant difference in the activity of those drugs against Gram-positive and Gram-negative isolates.

Graph 1: Antibiotic Susceptibility Testing



3.4 Multiple antibiotic resistance index:

The multiple antibiotic resistance (MAR) indexes of the isolated

Bacteria were determined with reference to ten different antibiotic used in this study. The values of MAR indexes are shown in Table 5. Analysis of the MAR index of isolates showed that all 5 isolated bacteria studied presented ratios above 0.2, indicating high resistance. A MAR greater than 0.2 means that the high risk source of contamination is where antibiotics are frequently used (Ayandele et. al., 2020)

MAR was calculated as the number of antimicrobials to which the isolate is resistant divided by the number of antibiotics to which the isolate is tested. Highest MAR Indices were detected in sample A and followed by sample E. Sample B, C, D had MAR index less than 0.3.

Table 5: Multiple Antibiotic Resistance (MAR) index (n=10)

	Sample No.				
	A	**B**	**C**	**D**	**E**
Number of antimicrobial to which isolate is resistant	6	2	2	2	4
Number of antibiotics to which the isolate is subjected	10	10	10	10	10
Multiple antibiotic resistance index (MAR)	0.6	0.2	0.2	0.2	0.4

4. Conclusion

Hands and mobile phones can act as a carrier for infectious agents, suggesting the need for proper hand hygiene and disinfecting mobile phones surfaces. The most important factor associated with mobile contamination was being unaware of the facts that mobile phones can harbour microorganisms and mobile phones need to be cleaned with antiseptics.Hand hygiene practice. Our results could help to raise awareness in our society about the importance of hand hygiene and frequently used devices, decreasing bacterial contamination and limiting the transmission of pathogens. Further study is recommended to identify the genetic diversity of the isolated bacteria and to determine the resistance genes of the multidrug resistant isolates.

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