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Vol. 10(8), pp. 254-259, 28 February, 2016
DOI: 10.5897/AJMR2015.7621
Article Number: 7E0456357469
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

The growth potential and antimicrobial susceptibility patterns of *Salmonella* species and *Staphylococcus aureus* isolated from mobile phones of food handlers and health care workers in Jimma Town, Southwest Ethiopia

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Received June 12, 2015; Accepted 31 August, 2015

Mobile phones are increasingly being used by all people in day to day life. However, they are found suitable breeding grounds for various pathogenic microorganisms. This study was aimed to determine the growth potential and antimicrobial susceptibility patterns of *Salmonella* species and *Staphylococcus aureus* isolated from mobile phones of food handlers and health care workers in Jimma Town, Southwest Ethiopia. Collection of mobile phone cotton swab samples and laboratory based microbiological analysis was used as the study design. A total of 188 mobile phones were sampled from food handlers and health care workers. The growth potential of *Salmonella* spp. and *S. aureus* isolated from mobile phones was assessed in various food items. The results have shown that *Salmonella* spp. and *S. aureus* isolated from mobile phones of food handlers and health care workers were found growing to their infective dose within 12 to 18 h in the sampled food items. Regarding the antimicrobial susceptibility test patterns, *Salmonella* spp. isolates were susceptible to ciprofloxacin, norfloxacin, gentamycin, chloramphenicol, and kanamycin, though they were highly resistant to ampicillin and nalidixic acid. On the other hand, *S. aureus* isolates were susceptible to gentamycin, chloramphenicol, amikacin, ciprofloxacin, streptomycin, and kanamycin. In multidrug resistance patterns, 5 and 6 drugs resistance were observed in *Salmonella* spp. and *S. aureus,* respectively. This indicates that mobile phones could play a significant role in spreading drug resistant infectious agents within the community. Therefore, the outmost care should be taken in using mobile phones.

Key words: Growth potential, microbial pathogens, mobile phones.

INTRODUCTION

Mobile phones are increasingly being used by all people in day to day life. They become in contact with various surfaces and are thus likely to be getting contaminated with various organisms (Tambe et al., 2012). Mobile phones make most human activities easier; however, they pose a number of serious public health problems as well (Czapiński and Panek, 2011).

Several pathogenic microbes including *Salmonella* species and *Staphylococcus aureus* have been isolated in different countries from mobile phones by many

researchers (Ekrakene and Igeleke, 2007; Akinyemi et al., 2009; Al-Abdalall, 2010). The presence of pathogenic microbes on mobile phones could indicate unknowingly that the devices had played a great role in spreading the infectious agents within the community and cause disease outbreaks (Akinyemi et al., 2009). The subscription of mobile phone technology is highly increased in today's world. It is estimated that in Ethiopia, approximately 40 million people have their own mobile phones, including adults and children. However, to the knowledge level of the investigator, there has been no published data on the growth potential of microbes isolated from mobile phones. Therefore, this study was aimed to determine the growth potential and antimicrobial susceptibility patterns of *Salmonella* spp. and *S. aureus* isolated from mobile phones of food handlers and health care workers in Jimma Town, Southwest Ethiopia.

METHODOLOGY

The study site and period

The study was conducted in Jimma town which is located at 353 km Southwest of Addis Ababa, Ethiopia. The geographical coordinates of the town are 7°41'N latitude, 36°50'E longitude (Abebe et al., 2011). The study was conducted from September 2012 to June 2013.

Study design and population

Collection of mobile phone swab samples and laboratory based microbiological analysis was used as the study design. A total of 188 mobile phone user samples including 119 health care workers and 69 food handlers were taken as the study population. The selection of study population participants was based on using purposive sampling technique. The sample size was determined using the statistical formula developed by Kothari (2004).

Sample collection

The sampled mobile phones were aseptically swabbed using sterile cotton moistened with normal saline solution by rolling it over exposed outer surface of the mobile phones. The cotton swabs were placed into a tubes containing 10 ml sterile normal saline and kept in ice box and transported to Research and Postgraduate Laboratory, Department of Biology, College of Natural Sciences, Jimma University for microbiological analysis. The microbiological analysis was done after two-three hours of sample collection following standard microbiological methods.

Inoculation and enumeration

Isolation of S. aureus

One milliliter of each mobile phone swab samples was transferred aseptically into 9 ml of buffered peptone water (BPW) and vortex mixed thoroughly for 5 min. The homogenates were serially diluted from 10^{-1} to 10^{-6} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates of Mannitol salt agar (MSA). The plates were incubated at 37°C for 24 h.

Identification of S. aureus

Golden yellow colonies from the MSA plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37°C for 24 h for further purification. Then, a loopful of culture from the nutrient broth was streaked on nutrient agar supplemented with 0.75% NaCl and again incubated at 37°C for 24 h. Finally, the distinct colonies were characterized using the established microbiological methods (Acco et al., 2003). Gram-positive cocci with cluster arrangement under the microscope were subjected to preliminary biochemical tests (coagulase, catalase, and oxidase).

Isolation of Salmonella spp.

To test the presence of *Salmonella* spp. in the sampled mobile phones, 1 ml cotton swab sample of each mobile phone was aseptically transferred into a tube containing 9 ml of buffered peptone water, homogenized for 5 min and then incubated at 37°C for 24 h for recovery of the organism (Primary enrichment). Following the buffered peptone water primary enrichment, 1 ml of the culture from the buffered peptone water was transferred into 10 ml of selenite cysteine broth (Oxoid) and was incubated at 43°C for 48 h (Secondary enrichment) (Johnson and Case, 2007).

Identification of Salmonella spp.

A loopful of suspension from selenite cysteine broth (Oxoid) tube was streaked onto Xylose Lysine Deoxycholate (XLD) agar plate (Oxoid) and incubated at 37°C for 18 h.

Characteristic black centered red colonies from the selective media were picked, further purified and biochemically tested (Triple iron sugar agar, Lysine iron agar, Simmons Citrate agar, Urea agar and SIM media) based on standard methods (Johnson and Case, 2007).

Determination of the growth potential

In order to standardize the procedure (*Salmonella typhi* ATCC13311 and *S. aureus* ATCC25923) were used as a control in this study. The growth potential of *Salmonella* spp. and *S. aureus* isolated from mobile phones was assessed in food item (Injera and Bread). 200 g of each food item was steamed at 80°C for 10 min to kill any vegetative cell, including *Salmonella* spp. and *S. aureus* which might be present in the items. Steamed food (10 g each) was examined for aerobic mesophilic bacteria and aerobic bacterial spores. Then, 100 g of each street food item was challenged separately with 1 ml overnight culture of the test strains to give an inoculums level of 10^2 to 10^3 cfu/g. To determine the initial inoculums level, 10 g of each freshly inoculated food was homogenized in 90 ml of BPW and 0.1 ml of appropriate dilution

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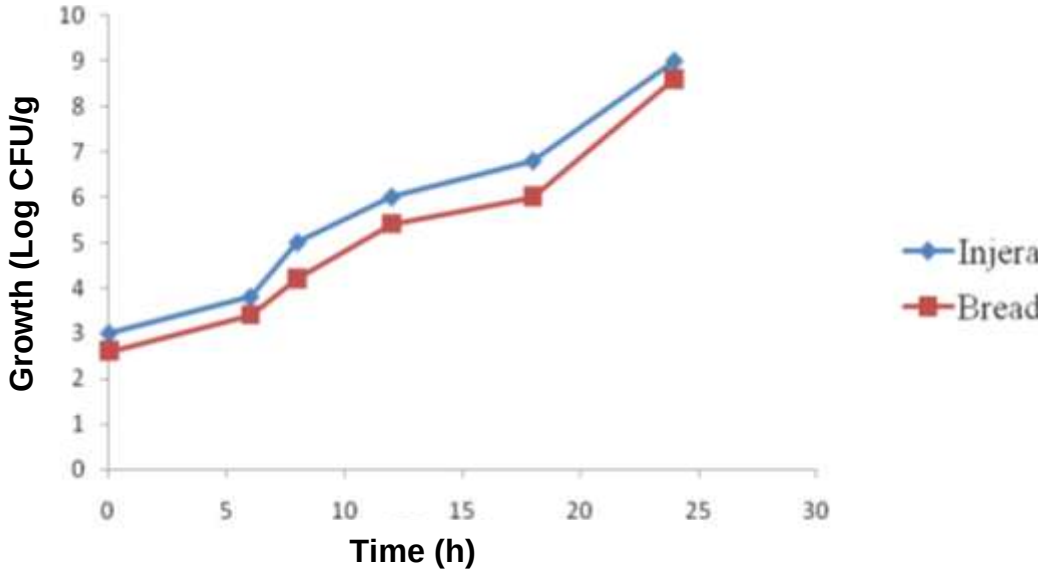

Figure 1. The growth potential of *S. aureus* isolated from mobile phones, Jimma town, Southwest Ethiopia.

was spread plated on XLD for *Salmonella* spp. and MSA for *S. aureus* agar plates to count *Salmonella* spp. and *S. aureus.* A portion of food sample was further sampled aseptically at 6 h interval from 0 to 24 h (Muleta and Ashenafi, 2001).

Antimicrobial susceptibility test for *Salmonella* spp. and *S. aureus* isolates

Antimicrobial susceptibility test for *Salmonella* spp. and *S. aureus* isolated from mobile phones was performed using the disk diffusion method in Mueller Hinton Agar (Oxoid). Briefly, a standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculums was matched with the turbidity standard of 0.5 McFarland (Bauer et al., 1966). The results were interpreted as per the criteria of the National Committee for Clinical Laboratory Standards Institute (Wikler et al., 2007). The isolates were categorized into resistance, intermediate, and susceptible based on their zone diameter measurements. The intermediates were considered as resistant in this study. Drug disk with their defined concentration, chloramphenicol (30 μgml^{-1}), ciprofloxacin (5 μgml^{-1}), clindamycin (2 μgml^{-1}), erythromycin (15 μgml^{-1}), gentamycin (10 μgml^{-1}), kanamycin (30 μgml^{-1}), penicillin (10 μgml^{-1}), amikacin (30 μgml^{-1}), streptomycin (10 μgml^{-1}), and tetracycline (30 μgml^{-1}) were used for *S. aureus* and ampicillin (10 μgml^{-1}), nalidixic acid (30 μgml^{-1}), kanamycin (30 μgml^{-1}), tetracycline (30 μgml^{-1}), chloramphenicol (30 μgml^{-1}), norfloxacin (10 μgml^{-1}), gentamycin (10 μgml^{-1}), ciprofloxacin (5 μgml^{-1}), and streptomycin (10 μgml^{-1}) were used for *salmonella* spp.

RESULTS

From a total of 188 mobile phone samples examined for microbiological safety, 41.5% of them were found positive for *S. aureus*. Over 22.5% of them were isolated from mobile phones of food handlers, whereas 19% were from health care workers mobile phones. On the other hand, 11.70% of the sampled mobile phones were positive for *Salmonella* spp. Specifically, *Salmonella* spp. was isolated from 6.38% of health care workers and 5.32% of food handler mobile phones.

In challenge studies, *S. aureus* isolates had increased by 1.5 Log CFU/g within 8 h in both food items (Bread and Injera). The growth rate in the first 8 h had shown steady increase and then finally reached counts of ≥9 Log CFU/g at 24 h (Figure 1).

Salmonella spp. isolates reached counts of ≥8 Log CFU/g within 24 h in both food items (Bread and Injera). There was about 1.2 Log CFU/g increase in the first 6 h and then a steady growth has been found thereafter. Relatively lower growth rate was observed in bread than in the injera (Figure 2).

Antimicrobial susceptibility test

Salmonella spp. isolates were susceptible to ciprofloxacin, norfloxacin, gentamycin, chloramphenicol, and kanamycin; though they were highly resistant to ampicillin and nalidixic acid (Table 1). In multidrug resistance pattern, 5 drugs resistance were observed in *Salmonella* spp. isolates.

S. aureus isolates were susceptible to gentamycin, chloramphenicol, amikacin, ciprofloxacin, streptomycin, and kanamycin. However, the isolates were found highly resistant to penicillin G and clindamycin (Table 2). In multidrug resistance pattern, 6 drugs resistance were observed in *S. aureus* isolates.

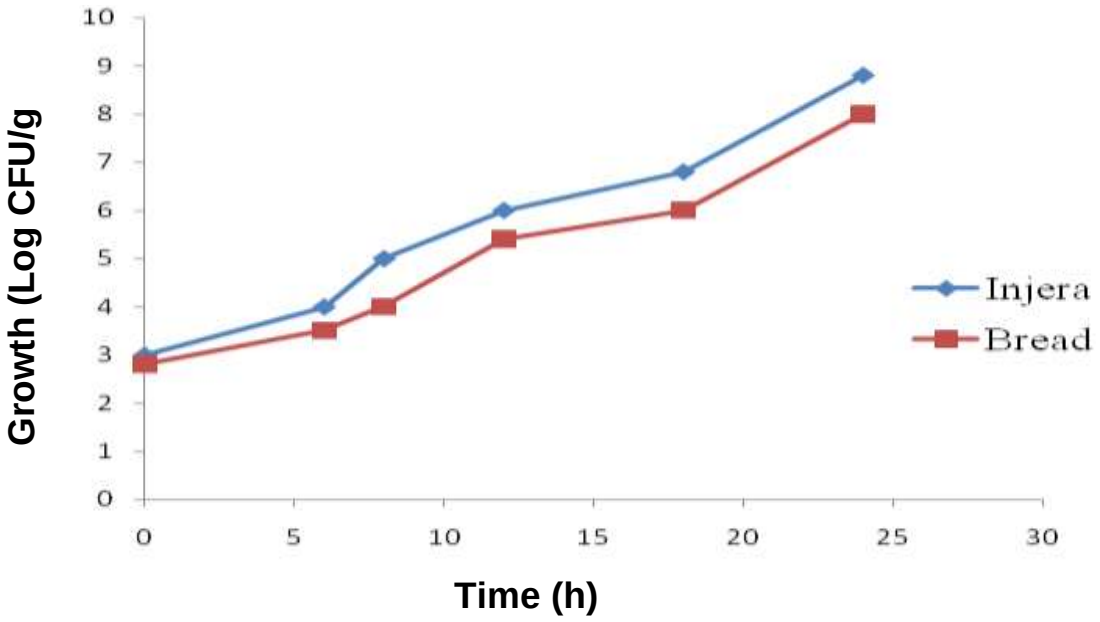

Figure 2. The growth potential of *Salmonella* spp. isolated from mobile phones, Jimma town, Southwest Ethiopia.

Table 1. Antimicrobial susceptibility patterns of *Salmonella* spp. isolated from mobile phones, Jimma town, Southwest Ethiopia.

Antimicrobial discs with defined concentration (µg)	Resistance (%)	Intermediate (%)	Susceptible (%)
Ciprofloxacin (5)	-	-	22 (100)
Ampicillin (10)	21 (95.5)	-	1 (4.55)
Chloramphenicol (30)	-	2 (9.09)	20 (90.91)
Nalidixic acid (30)	18 (81.82)	2 (9.09)	2 (9.09)
Kanamycin (30)	-	3 (13.64)	19 (86.36)
Norfloxacin (10)	-	-	22 (100)
Gentamycin (10)	-	1 (4.55)	21 (95.45)
Tetracycline (30)	6 (27.27%)	-	16 (72.73)
Streptomycin (10)	15 (68.18)	3 (13.64)	4 (18.18)

Table 2. Antimicrobial susceptibility patterns of *S. aureus* isolated from mobile phones, Jimma town, Southwest Ethiopia.

Antimicrobial discs with defined concentration	Resistance (%)	Intermediate (%)	Susceptible (%)
Gentamycin(10)	-	2 (2.6)	76 (97.4)
Erythromycin(15)	31 (39.7)	34 (43.6)	13 (16.67)
Chloramphenicol(30)	1 (1.3)	2 (2.6)	75 (96.1)
Ciprofloxacin(5)	3 (3.8)	2 (2.6)	73 (93.6)
Amikacin(30)	1 (1.3)	2 (2.6)	75 (96.1)
Kanamycin(30)	2 (2.6)	6 (7.7)	70 (89.7)
Streptomycin(10)	-	6 (7.7)	72 (92.3)
Penicillin G (10)	78 (100)	-	-
Tetracycline(30)	21 (26.9)	32 (41.035)	25 (32.05)
Clindamycin(2)	57 (73.1)	17 (21.8)	4 (5.1)

DISCUSSION

The current challenge studies conducted using *Salmonella* spp. isolated from mobile phones of food handlers and health care workers revealed that the isolates grew to their infective doses (≥5 Log CFU/g) in bread and injera samples within 12 and 18 h, respectively. The maximum counts recorded were ≥8 Log CFU/g in both food items (Bread and Injera) within 24 h.

Likewise, report from Addis Ababa Ethiopia made by Muleta and Ashenafi (2001) indicated that the mean counts of *Salmonella* isolates reached >8 Log CFU/g within 24 h. On the other hand, Erku and Ashenafi (1998) evaluated the growth potential of *Salmonella* spp. in weaning foods in Addis Ababa, Ethiopia where *Salmonella* had grown to approximately 4 Log CFU/ml within 8 h and reached counts as high as Log 8 CFU/ml within 12 h.

S. aureus isolates tested in the present study had reached mean counts≥6 Log CFU/g in both food items (Bread and Injera) within 12 and18 h, respectively. The infective dose for *S. aureus* is 6 Log CFU/g. This is in agreement with the study reported by Muleta and Ashenafi (2001) from Addis Ababa Ethiopia. *S. aureus* toxin is produced when the count exceeds 6 Log CFU/g. The maximum growth of *S. aureus* (9 Log CFU/g) was observed in the current study within 24 h. In general, the trends of growth of the test strains were similar with increasing pattern almost throughout the observation period. *Salmonella* spp. isolated from mobile phones indicates marked resistance to commonly used antibiotics, such as ampicillin (95.45%), nalidixic acid (81.82%), and streptomycin (68.18%). Similarly, in a study conducted in Bangladesh, Ahmed et al. (2011) reported higher frequency of *Salmonella* spp. resistant to ampicillin and nalidixic acid. In the current study, however, *Salmonella* spp. isolates were found sensitive to ciprofloxacin (100%), norfloxacin (100%), gentamycin (95.45%), chloramphenicol (90.91%), and kanamycin (86.36%). Likewise, the highest frequency (100%) of sensitivity to chloramphenicol was reported in earlier studies from India (Cailhol et al., 2005; Nesa et al., 2011). *Salmonella* spp. isolate has shown resistance against five antibiotics in this study which were considered as multidrug resistant strains (Sivakumar et al., 2012). The major reasons for the presence of multidrug resistance among *Salmonella* spp. is due to mutability of bacteria and inappropriate use of antibiotics (Ochman et al., 1996). Many people purchases antibiotics in the open market without any medical prescription and use them for the wrong diseases and infections (Tagoe and Attah, 2010).

S. aureus resistance to antibiotics such as penicillin g (100%), clindamycin (94.4%), tetracycline (76.9%), and erythromycin (74%) in this study is in agreement with the study by Tambekar et al. (2008) from India where the highest frequency of resistance was recorded for antibiotics penicillin, erythromycin, and tetracycline. This indicates that there might be fast growing public health threat within the community. Therefore, it requires strong controlling system of the personal hygiene and educating food handlers and health care workers regarding microbial contamination of mobile phones. Mohamad et al. (2010) from Cairo reported that, *S. aureus* isolated from mobile phones of health care workers demonstrated the highest frequency of resistance to several antimicrobials. This may be due to indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, self-medication, and inappropriate use of antibiotics (Tagoe and Attah, 2010). *S. aureus* isolate had shown multidrug resistance against six antibiotics. This could be the major challenge for treating staphylococcal infections.

In conclusion, *Salmonella* spp. and *S. aureus* isolated from mobile phones of food handlers and health care workers in Jimma town, Southwest Ethiopia were found to be able to grow to their infective doses within 12 to18 h in both food items (Bread and Injera). In addition, both *Salmonella* spp. and *S. aureus* isolates had shown the highest frequency of resistance for most of the antimicrobials tested. This indicates that mobile phones could play a significant role in spreading drug resistant infectious agents within the community. Therefore, the outmost care should be taken in using mobile phones.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors acknowledge Jimma University for funding.

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