

# Journal of Microbiology and Antimicrobials

Volume 6 Number 4, June 2014

ISSN 2141-2308



*Academic  
Journals*

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). *Microbiology: Concepts and Applications*. McGraw-Hill Inc., New York, pp. 591-603.

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# Journal of Microbiology and Antimicrobials

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## ARTICLES

**Yeasts in the mammary environment of the cattle in the region of Sidi M'hammed Ben Ali, Wilaya of Relizane, Algeria**

Akdouche L., Aissi M., Zenia S. and Saadi A.

**Antibiotic-resistant *Staphylococcus aureus* isolated from mobile phone and hands of Health care workers in the Hawassa referral Hospital, South Ethiopia**

Deresse Daka

Full Length Research Paper

# Yeasts in the mammary environment of the cattle in the region of Sidi M'hammed Ben Ali, Wilaya of Relizane, Algeria

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Received 7 January, 2014; Accepted 20 May, 2014

**Mastitis is one of the principal pathologies in the dairy bovine exploitation. Most of the cases are caused by bacteria, but some are caused by fungi. The objective of our study was to evaluate the occurrence of these fungi in mammary glands of 39 cows (mastitic and clinically healthy cows) belonging to two farms (four exploitations using manual milking and three exploitations using milking machine) in the area of Sidi M'hammed Ben Ali, Wilaya of Relizane and to assess some risk factors (the tubes of drug, animal excretion, goblets-milkers, the milkers' hands and the litter). For this purpose, 150 samples of milk and 94 swabs were used. Our results reveal the presence of a heavy load of fungi cells in healthy and mastitic milks, with a strong frequency of *Trichosporon* sp. (43, 58%) followed by *Candida* sp. (30.76%). The same yeasts were isolated from swabs.**

**Key words:** Mastitis, fungi, antibiotics, milking machine, the milker, Algeria.

## INTRODUCTION

Mycotic mastitis was described from the beginning of the last century (Klein, 1901). This mastitis aroused some skepticism and numerous debates because the incriminated agents are often contaminants of the outside or the common saprophytes. Although still inadequately known, they seem to draw the attention of pathologists, especially since the acceptance of everyday treatment (intra-mammary antibiotic). The rates of the observed mycotic mastitis vary from 0.34 (Loftsgard et al., 1960) to 3.9%. Swinne-Desgain (1971) and Fortier (1990) said yeasts are responsible for 1.76 % cases of mastitis (clinic and sub-clinic). Milk from a healthy udder does not

contain either mushrooms or bacteria. It is better to speak about a fungal basic flora, resulting from the environment (dust resulting from feeds, equipment of collection as well as those of animals and even man). It is very common to find in the unpasteurized milk yeasts of the genre, *Candida* and mold, *Penicillium*, which can alter some dairy products.

Mycotic mastitis is split into two big groups according to the moment of appearance: primary mycotic Mastitis (bacterial preliminary mastitis) and secondary mycotic mastitis. The latter appears often straightaway, without antibiotic treatment or generally follows a bacterial

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**Table 1.** Sampling plan.

Nature of the sampling	Number of samples		
	Manual milking	Milking machine	Total
Number of cows	17	22	39
Numbers of milk samples	65	85	150
Numbers of anal swabs	17	22	39
Numbers of vaginal swabs	13	22	35
Number of swabs	0	2	2
Number of swabs on the hands of the milker	3	0	3
Number of swabs on creams Antibiotics	12	0	12
Numbers of litter samples	0	3	3

mastitis or an intramammary administration of antibiotics by diathétique way. According to some authors (Bertslinger et al., 1964), the first ones would represent 30% cases and the second 70% cases.

In Algeria, very few studies have been done on the prevalence of the fungal mastitis in the dairy bovine farms as well as on various factors favoring their appearance and development (Mebarki, 2007; Ksouri, 2008). So, the objective of the study is to determine the prevalence of mastitis caused by yeasts and to know the number of risk factors in some dairy bovine farms in Relizane.

## MATERIALS AND METHODS

### Distribution of a questionnaire

Pre-investigation was done in the last quarter of the year 2007 and the first half of 2008 to estimate the epidemiological situation of this pathological entity within the dairy beef herd in the region of Relizane. For that purpose, a questionnaire was distributed to the veterinarian practitioners. This investigation is on the breeding technique, frequency of the clinical mastitis in this breeding and the percentage use of antibiotics in the treatment of clinical mastitis.

### Choice of farms

Four dairy farms with manual milking and tree with machine milking were used in this study. This selection was based on the comparison of both types of milking. All the farms exist in the same region- Sidi M'hammed Ben Ali, Wilaya de Relizène.

### Nature and number of samples

A total of 244 samples were collected by the veterinarian practitioners of Sidi M'hammed Ben Ali's region. That is 150 samples of milk taken from 39 existing cows in 7 farms. The samples were obtained with different mammary glands health status: 19 cows with healthy mammary glands, 15 cows with subclinical mastitis as determined by the California Mastitis Test (CMT) and 05 cows with clinical mastitis, defined as follows: swelling, reduced milk flow and abnormal milk appearance, fever, inappetence, ataxia. CMT was used to identify subclinical mastitis on mammary gland of the cows. For this study, milk samples from gland affected with subclinical mastitis were included when the reaction to CMT was at least grade 1. This corresponds with an

appearance of viscous milk that does not adhere to the bottom of CMT plate, and correlates with 400,000-1,500,000 somatic cells/ml (Scott et al., 1986) (6 milk sampling emptied of their tube because they were badly kept), 91 swabs [39 anal swabs and 35 vaginal swabs of which four were badly kept), two swabs on milking machine, 3 on the hands of the milkers and 12 swabs of antibiotic creams. And at the end, three samples of litter were got back.

From every cow during lactation, four takings of milk (a taking of milk of every trayon), an anal swab, and a vaginal swab were taken once during all the period.

In every breeding with manual milking, swabbing was done by the hands of the milker before the milking (factor of contamination), there was recovery of the tubes of antibiotic cream (factor of release) used for the treatment of cows clinically and a sample of the litter was collected just before its renewal (factor of enrichment). Some takings were made in the breeding with machine milking except swabbing of the milkers' tumblers of the milking machine (factor of contamination) (Table 1).

### Milk sampling

The correct realization of the sampling procedure was a necessity, in terms of the ubiquity of fungi which can contaminate the milk. The characteristics of the surrounding atmosphere were noted. The cows' environment was not loaded with dust (hays moving nearby and agitated animals). If such was the case, the animals of the dusty premises must have gone out. The milk sampling was realized according to the protocol of Guerin and Guerrin-Fauble (2007), which consists of washing the milkers' hands with a disinfecting soap, identifying the flask (in wide opening) with indelible felt-tip: number of the cow, the mammary gland quarter (FR, FL, RR or RL), date and time. The udder was carefully washed and wiped; the rough drafts of milk for rinsing the canal of the udder were eliminated (not more than 2 jets, otherwise there would be risk of the taking having germs). The teat canal of the udder was disinfected with a compress soaked with alcohol at 70°C; the sterile flask was opened to maintain the openings managed downward. The rubber was kept in the same hand without touching the inside; some milliliters of milk were taken and recorded in the flask. Every udder was disinfected before taking the milk of the corresponding district. Finally, the takings of milk were kept in - 20°C until the day of their analysis.

### Mycological analyses of the milk

Mycological analysis was realized in the laboratory of Parasitology - Mycology of the Veterinary Graduate School- Algiers. It consists of a direct examination of the samples of milk, after vital staining. Milk

samples were centrifuged and the sediment was inoculated on the surface of Sabouraud Dextrose Agar (SDA) (QUELAB, Laboratories INC and code: QB-39-3806) added of chloramphénicol (QUELAB, Laboratories INC and code: QB-39-3806) and incubated for 3 days at 25 °C. Finally, isolated yeasts and filamentous fungi were identified using microscopic characterization. Yeasts isolated were identified by the gallery Pasteur (gallery Auxanogramme) (DIMED, code: 15300Algeria). This identification was performed taking into consideration morphological characteristics, like formation of chlamydoconidium, pseudohyphae and germinal tube development. This gallery is composite due to its various cultural middle and various tests for the precise identification of yeasts.

1. Middle Sabouraud/Chloramphénicol at 37°C: this test allows one to highlight the potential pathogenic character of the yeast when it develops in a temperature, bordering the corporal temperature.
2. Middle Sabouraud/Actidione; this test allows one to highlight colonies sensitive to Actidione (Cycloheximide). Colonies having grown on this middle are considered resistant to Actidione (R); colonies not having grown on this middle are considered sensitive to Actidione (S).
3. Middle with cream of rice (rice cream): this middle favors the production of chlamydoconidia characteristics of *Candida albicans* in anaerobic middle.
4. Middle with serum for blastèse: the serum of bovine is used as middle to favor the production of *Candida*'s typical germinal albicans tubes (test of germination).
5. Middle in the urea Indole: this test allows one to look for the hydrolysis of the urea. The change of the middle colour of yellow-orange to purple-red corresponds to the secretion of an uréase. The yeast which turns the middle to red in 4 h is *C. neoformans*.

A quantitative search for mushrooms (counting of colonies) and qualitative search (the various tests for identification) are made. For the identification of the genre and species of yeasts, the key of identification of yeasts proposed by Drouhet and Dupont (1985) was used.

### The anal, vaginal swabs and the material of milking

The vaginal and the anal excretions were collected by swabbing in the perineum and vaginal regions. Swabbing of goblets was done-milkers of milking machines only in two dairy cow farms and swabbing of the hands of the milking men before the milking.

Swabbings were made by direct scattering of the swab on the surface of the SDA plates added to chloramphenicol. After incubation for 3 days at 25°C, the colonies of yeasts were identified as previously.

### The litter samples

The collected litter was deposited in one sterilized conical glass cup containing sterile physiological water, then the whole was homogenized and the rest was left for 30 min. Some gouts of the sediment are then inoculated on SDA added to chloramphénicol. Cultures were incubated for 3 days at 25°C.

## RESULTS

### Results of the questionnaire distributed to the veterinarian practitioners

The veterinarians in charge of the follow-up of the bovine breeding note that the measures of hygiene are absent.

Indeed, 60% of the milkers do not disinfect their hands before and after every milking. The udder is not disinfected before the milking in 45.71% cases. The majority of the breeding are done in hindered stall (54.85%); 57.14% of the breeders use the same rag for the disinfection of the udder and in 65.71% cases, this rag is not disinfected after each use; 25.71% of the farmers disinfect their milking material once a week; 17.14% of the breeders change the cow litters only once a week.

This report thus incited us to start a study on prevalence of the mastitis of fungal origin.

### Results of the mycological analysis of the samples collected

Of the 244 samples realized, 91 are positive, that is, 37.3% and 78 fungal species were identified: 35 species in the breeding with manual milking namely *Candida sp* (25.7%), *Trichosporon sp.* (48.6%), *Rhodotorula sp.* (8.6%), *Cryptococcus sp.* (2.9%), *Torulopsis sp.* (2.9%), *Penicillium sp.* (8.6%), *Aspergillus sp.* (2.9%) (Table 4); 43 species in the breeding with machine milking namely *Candida sp.* (34.9%), *Trichosporon sp.* (39.5%), *Rhodotorula sp.* (9.3%), *Cryptococcus sp.* (4.6%), *Torulopsis sp.* (2.3%), *Penicillium sp.* (7%) and *Aspergillus sp.* (2.3%) (Table 2).

## DISCUSSION

Fungal cultures were observed in 68 samples of milk (Table 3). A study was done on the mammary infection of dairy cows in Sidi M' Hammed Be Ali's region, Wilaya of Relizane, from December 2007 to May 2008.

In our survey, the mycological examination of the milk samples and the realized swabs showed the presence of yeasts and filamentous fungi, with a higher frequency of yeasts (Table 3). This is in line with that of the literature. Indeed, the most frequent yeasts genus were *Candida* (30.76%) and *Trichosporon* (43.58%) (Table 4). Many authors noted that the fungal bovine mastitis is predominantly caused by yeasts (Swinne-Desgain, 1971; Kuo and Chang, 1993; Aalbaek et al., 1994; Watts, 1988; Lagneau et al., 1996; dos Santos and Marin, 2004).

The mycological analysis also revealed that, the same genre of yeasts was found in both types of exploitations namely *Candida*, *Trichosporon*, *Rhodotorula*, Cryptocoques and *Torulopsis* (Costa et al., 1993; Krukowski et al., 2001; Krukowski et al., 2006); with a higher frequency for *Candida* and *Trichosporon* genres (30.76; 43.58%) and then *Rhodotorula* (7.69%) and Cryptocoques (3.84%) (Table 4). All these fungal agents, with the exception of *Rhodotorula* have been detected before as pathogenic agents in numerous inquiries on fungal mastitis (Moulinier, 2003). Prevalence of the

**Table 2.** Various species of yeasts and molds isolated.

Genre	%	Species	Number
<b>Breeding with manual milking</b>			
	25.7	<i>Candida zeylanoides</i>	1
		<i>Candida pseudotropicalis</i>	5
<i>Candida</i> sp.		<i>Candida guilliermondii</i>	1
		<i>Candida tropicalis</i>	1
		<i>Candida parapsilosis</i>	1
<i>Trichosporon</i> sp.	48.6	<i>Trichosporon cutanium</i>	10
		<i>Trichosporon capitatum</i>	7
<i>Rhodotorula</i> sp.	8.6	<i>Rhodotorula rubra</i>	3
<i>Cryptococcus</i> sp.	2.9	<i>Cryptococcus terreus</i>	1
<i>Torulopsis</i> sp.	2.9	<i>Torulopsis pulcherrima</i>	1
<i>Penicillium</i> sp.	8.6	<i>Penicillium</i> sp	3
<i>Aspergillus</i> sp.	2.9	<i>Aspergillus</i> sp	1
Total	100		35
<b>Breeding with machine milking</b>			
	34.9	<i>Candida zeylanoides</i>	1
		<i>Candida pseudotropicalis</i>	2
<i>Candida</i> sp.		<i>Candida guilliermondii</i>	7
		<i>Candida tropicalis</i>	4
		<i>Candida lusitanae</i>	1
<i>Trichosporon</i> sp.	39.5	<i>Trichosporon cutanium</i>	8
		<i>Trichosporon capitatum</i>	6
		<i>Trichosporon fermentens</i>	3
<i>Rhodotorula</i> sp.	9.3	<i>Rhodotorula glutinis</i>	4
<i>Cryptococcus</i> sp.	4.6	<i>Cryptococcus terreus</i>	2
<i>Torulopsis</i> sp.	2.3	<i>Torulopsis glabrata</i>	1
<i>Penicillium</i> sp.	7	<i>Penicillium</i> sp	3
<i>Aspergillus</i> sp.	2.3	<i>Aspergillus</i> sp	1
Total	100		43
Total			78

**Table 3.** Frequency positive samples according to the milking procedure.

Milking procedure	Number of milk samples	Positive samples	Frequency (%)
Manual milking	65	30	46.15
Machine milking	85	38	44.70
Total	150	68	45.3

fungal mastitis varies from 1 to 44% according to authors' number (Loftsgard et al., 1960; Monga et al., 1971; Swinne-Desgain, 1971; Farnsworth et al., 1972; Kumer et al., 1975; Fenizzia et al., 1976; Awad et al., 1980; Ramisse et al., 1982).

Global frequency observed on the present study was considered at 45.33% for the exploitations with clinical mastitis and subclinical mastitis (Table 3), which is similar to those of Swinne and Desgain (1971). This frequency may be explained by the animal management put in place in the visited dairy farms in the region of Sidi M'hammed Ben Ali (results of the questionnaire).

The genus *Trichosporon* was quoted by several authors as being a potential pathogenic fungus; in particular, *T. capitatum*, and *T. cutaneum* (Loftsgard et al., 1960; Fameree et al., 1970). The present study highlighted these species with a 43.58% rate (23.06% for *T. cutaneum*, 16.66% for *T. capitatum*), higher than the rates found in a survey done by Mebarki (2005) in Algiers (19.25 %) on dairy exploitations of subclinical mastitis. Other authors pointed to lower rates, such as Moretti et al. (1998), who isolated *T. capitatum* in 31.2% cases and *T. cutaneum* in 18.72% cases in Italy. Aalbaek et al. (1994) described five cases of mastitis caused by *Tr*



**Table 4.** Frequency of fungi isolations in the samples.

Genre	Percentage (%)
<i>Candida</i> spp.	30.76
<i>Trichosporon</i> spp.	43.58
<i>Rhodotorula</i> spp.	8.97
<i>Cryptococcus</i> spp.	3.84
<i>Torulopsis</i> spp.	2.56
<i>Penicillium</i> spp.	7.69
<i>Aspergillus</i> spp.	2.56

**Table 5.** Summary of the number of yeasts and filamentous fungi isolated from milk.

Fungi	Manual milking	Machine milking
Yeasts	40	46
Moulds	08	06
Total	48	52

*capitatum* in Denmark, which is lower than that of this present result (13 cases). Costa et al. (1993) have described 21 mastitis cases caused by *T. cutaneum* in Brazil.

Concerning the genus, *Candida*, its strong predominance (30.76 %) in the whole of the positive samples confirms the importance of this yeast, often evoked as the main genus in the etiology of mycotic mastitis (Fameree et al., 1970; Farnsworth et al., 1972; Richard et al., 1980; Yeh et al., 1988; Kuo and Chang, 1993; Aalbaek et al., 1994; Lagneau et al., 1996; dos Santos et al., 2004). This frequency of *Candida* isolation was lower than that recorded in the region of Algiers by Mebarki (2005) (52.07%) and in the South of Brazil by Spanamberg et al. (2008) (37.9%), but superior to that (17.3%) noted by Sailor et al. (2004) in Brazil.

Prevalence of the fungal mastitis according to the milking modality was almost the same: in the manual milking, it is 46.15% and in the machine milking, it is 44.70%. This means that there is independence between the positive milk samples and the milking procedure at the beginning. The difference is not significant ( $p > 5$ ). The Chi-square test of independence was used for the comparison of both methods (manual milking and machine milking). This indicates that the problem does not settle at the level of the method of milking but in the conditions of the milking progress (the factors of enrichments, factors of releases and factors of contamination) (Table 5).

## Conclusion

The frequency of fungal mastitis is underestimated in Algeria. The present study shows cases of fungal mastitis found in two types of exploitations (manual milking or

machine milking). The isolation of the same genus of fungi in an almost similar percentage in both milking systems confirms the idea. This leads one to conclude that the problem of the fungal mastitis is not only connected to the milking modality but is connected to the conduct of farmers and the hygienic practices applied during the milking. The hygiene practices in the stables of the dairy farm do not have to be an additional act in the conduct of the farmers but a regular component of the farm management. With the aim of limiting the increase of the fungal mastitis, it is important to establish a specific diagnosis on healthy and pathological milk to modulate a treatment according to the etiology and clinical aspect of mastitis.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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## Full Length Research Paper

# Antibiotic-resistant *Staphylococcus aureus* isolated from mobile phone and hands of Health care workers in the Hawassa referral Hospital, South Ethiopia

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Received 26 January, 2014; Accepted 17 June, 2014

A swab of mobile phone and hands of health care workers (HCWs) were examined to determine the prevalence of *Staphylococcus aureus* (SA), different antibiotic resistant pattern were determined in a cross-sectional study design. The objective of this study was to isolate *S. aureus* from samples of mobile phone and hands of HCWs from Hawassa Referral Hospital and to determine their antibiotic resistant patterns. A cross-sectional study design was carried on 152 mobile phones and hands of HCWs from different rooms of Hawassa Referral Hospital and screened for the presence of *S. aureus*. Gram staining, oxidase, catalase, DNase, haemolysis and coagulase tests were employed for bacterial identification. 97.4% of the samples were contaminated with different microorganisms. However, the contamination level of *S. aureus* in mobile phone and hands of HCW's were 53.9 and 55.5%, respectively. A total of 82 *S. aureus* isolates from mobile phone and 84 *S. aureus* were obtained from hands of HCWs during this study. The levels of contamination with *S. aureus* were slightly higher in hands of HCWs. About 65.9 and 47.6% strains were resistant to Ampicillin (AP) (10 µg) and Penicillin G (PG) (10 µg), respectively. About 26.8, 31.7, 15.9, 40.2, 26.6, 22.0, 14.6, 40.2 and 31.7% strains were resistant to Amoxicillin (Ax) (30 µg), Ciprofloxacin (CIP) (5 µg), Ceftriaxone (CRO) (30 µg), Oxacillin (Ox) (1 µg), Chloramphenicol (CAF) (10 µg), Doxycycline (DOX) (30 µg), Gentamycin (10 µg), Vancomycin (V) (30 µg) and Tetracycline TTC (30 µg), respectively. The resistant level of *S. aureus* to CIP, CRO, CAF, DOX and Ax were low as compared to AP, PG, V and Ox. *S. aureus* is normally resident in different habitat; therefore, the *S. aureus* present in the mobile phone and hands of HCW's may have resulted from contamination of hands of HCWs, showing the need to improve personal hygiene conditions in the hospital, specially OR and ICU. The training of healthcare workers on strict infection control procedure, hand hygiene, mobile phone cleaning habit and environmental disinfection are standards to control pathogen transmission.

**Key words:** Antibiotics, *S. aureus*, mobile phone, cell phone, health care workers (HCWs).

## INTRODUCTION

Nosocomial infection increases gradually and causes a significant rate of mortality and morbidity. The etiological

agents of hospital acquired infections may spread through the hand of healthcare workers (HCWs),

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thermometers, stethoscopes, and even toys in the pediatric intensive care units of hospitals (Fleming and Randle, 2006).

Today, mobile phones have become one of the essential accessories of professional and social life. The use of cell phones often occurs in hospital halls, laboratories, and/or intensive care units (ICU) when dealing with severe illnesses (Brady et al., 2006).

*Staphylococcus aureus* is a global human pathogen and a common cause of invasive and life threatening infections. It is the most common cause of folliculitis (infection of the hair follicle), boils, furuncles and carbuncles, community associated cellulitis (Brook and Frazier, 1995; Diekema et al., 2001) endocarditis (Hoen et al., 2002), and is a common cause of bacteremia (Diekema et al., 2001; Javaloyas et al., 2002; Weinstein et al., 1997). Also, *S. aureus* can cause postoperative wound infections, food poisoning, pneumonia in infants, debilitated individuals and immunocompromised patients. *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant  $\beta$ -lactams (e.g. Methicillin and Oxacillin), the most commonly used class of antibiotics for skin infection. These strains were termed 'methicillin resistant *S. aureus*, or MRSA, a term that implied cross-resistance to all  $\beta$ -lactams including all penicillins and cephalosporins.

Staphylococci are normal flora of the skin and mucous membranes of animals and humans. Most pathogenic strains are usually coagulase-positive and have been found to cause disease in their hosts throughout the world (Larsen et al., 2000; Matsunaga et al., 1993).

Determination of levels of *S. aureus* and an evaluation of the antibiotic-resistant phenotypes of the isolates from mobile phone and hands of HCWs could serve as a tool for determining the hygiene standards implemented during handling of mobile phone at health sector. Data on antibiotic resistance could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the health service contaminated tools and its products. The aim of this study was to isolate *S. aureus* from mobile phone and hands of HCWs and further characterize their susceptibility patterns to eleven selected antibiotics.

## MATERIALS AND METHODS

### Study area, design and population

A cross-sectional study was conducted from August 2013 to December 2014 to determine the bacterial species from mobile and hands of HCWs in Hawassa town. A total of 152 mobile phones were randomly sampled from medical wards, laboratory rooms, ICU and operating rooms of senior doctors, general practitioners, doctors, nurses, laboratory technologists and other healthcare workers and were screened. This study area is situated in the southern part of Ethiopia at 250 km from the capital city of the country. A sample of size 152 was determined using sample size calculator in EPI info by setting CI at 95%, margin of error at 3% considering magnitude of mobile phone contamination to be 95%

from a previous study. Also, pre tested questionnaire was used to determine some socio-demographic and ring using rate and cleaning habit of the mobile phone (Fatma et al., 2009).

Total collected samples culture was subsequently obtained from the dominant hand of participants and their mobile phones at the same time. Gender, profession and duration of their profession, ring use, dominant hands of HCWs, routine cleaning of the mobile phones was recorded on pre tested questionnaire.

A sterile swab moistened with sterile saline was rotated over the surface of both sides of mobile phones; second swab was rubbed over the entire ventral surface of the dominant hand (including ventral surfaces of the thumb and the fingers) of HCWs.

### Identification of bacteria

A large colony with a convex, creamy appearance, pigmented white to golden yellow was isolated from a culture media. Gram staining was performed (Cruikshank et al., 1975) and Gram-positive cocci that occurred in clusters under the microscope were subjected to preliminary biochemical tests (the catalase, coagulase and oxidase tests). The identities of the isolates were confirmed based on positive results for the DNase test, beta-haemolytic patterns on blood agar enriched with 5% (v/v) sheep blood and the coagulase slide test for *S. aureus* using the (PROLD Diagnostics, Canada). The slide agglutination test was performed according to the manufacturer's instructions. Briefly, cells from a pure colony were placed on the clean area of the slide using a sterile toothpick and a drop of the PROLD reagent was added. These were mixed using the toothpick and the isolates were identified based on the formation of agglutination. An isolates that formed agglutination were recorded as *S. aureus* and maintained at 4°C in 30% glycerol for further characterization by antibiotic susceptibility testing.

### Antibiotic susceptibility

Antibiotic susceptibility tests were performed on all *S. aureus* isolates to determine their antibiotic-resistance profiles (Kirby et al., 1966). Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100  $\mu$ L) from each isolate suspension was spread plated on Mueller Hinton agar (supplied by Oxoid Company). Susceptibilities of the isolates to a panel of eleven different antibiotic discs (6  $\mu$ m in diameter, Mast group LTD MERSEY SIDE, UK) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18 - 24 h (NCCLS, 1999). Inhibition zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards (NCCLS, 1999) were used to interpret the results obtained. *S. aureus* isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic. Multiple antibiotic resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics. By direct colony suspension method, 0.5 McFarland equivalent inoculum were prepared in normal saline from 18-24 h agar plate culture. The suspension was further diluted to achieve desired inoculum concentration of 105 CFU/ml. All strains were spotted onto gradient plates. Plates were incubated overnight at 37°C for any visible growth. Readings were taken according to NCCLS guidelines (NCCLS, 1999).

## RESULTS

### Prevalence of *S. aureus* isolated from mobile phone and hands of HCWs

The prevalence of *S. aureus* isolates from mobile phone

**Table 1.** The source of sample and *S. aureus* result in the respective work place (n = 152).

Place of work	Profession					Student*	<i>S. aureus</i> result; No. (%)	
	Cleaner	Laboratory worker	Dr. (general practitioner)	Dr. (senior) specialist	Senior nurse		Mobile phone	Hands of HCWs
Wards	2	-	1	2	33	7	26 (31.7)	30(35.7)
Laboratory	4	33	-	-	-	-	25(30.5)	28(33.3)
OPD	2	-	8	4	22	6	20(24.4)	25(29.8)
ICU	1	-	1	2	4	1	3(3.7)	1(1.2)
OR	4	-	2	2	9	2	8(9.8)	0(0.0)
Total	13	33	12	10	68	16	82	84

\*Medicine, nurses, midwifery, laboratory technology and other health care givers.

and hands of HCW's were 53.9 and 55.3%, respectively. However, the rate of bacterial contamination of mobile phones was 97.4%. Among the contaminated bacteria species isolated from mobile phone and hands of the HCWs was *S. aureus*, coagulase negative *Staphylococcus* (CoNS), *Streptococcus* spp., *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* spp., *Citrobacter* spp., *Shigella* spp. and *Pseudomonas aeruginosa*.

The species identified from both sources were similar. Some of them are known to cause nosocomial infections. It was found that 50.6% of phones grew one bacterial species, 24.0% two different species, 22.8% three or more different species and no bacterial growth were identified in 2.6% of phones. Thus, *S. aureus* strains isolated from mobile phones and hands of HCW's were 53.9 and 55.3%, respectively. These all organisms isolated from different room workers such as medical wards, OPD, ICU, OR, Laboratory and other classes.

About 35.7% of the isolates were obtained from hands of HCW's who work in wards. Meanwhile, 31.7% were form mobile phone swabs at wards

(Table 1). The rate of routine cleaning of HCWs mobile phones was 5.3%, which means 94.7% of the participants never cleaned their mobile phones either daily or weekly. Although the laboratory technologists and nurses' phones have higher colony count, there was no significant difference in the rates of specific types of bacterial growth and colony counts isolated from all groups' mobile phones (Table 1).

About 28.3% of the entire study population had at least one ring on their finger. The mean colony count was higher in ring using staff's phones but there was no significant difference between rate of contamination and colony count. A total of 299 potential isolates were sub-cultured and further analyzed. However, only 82 for mobile phone and 84 for hands of HCWs isolates satisfied all the identification criteria and were used for subsequent analysis. These constituted a total of 82 *S. aureus* isolates for mobile phone and 84 isolates for hands of HCWs. The *S. aureus* isolates obtained from both source give a prevalence of *S. aureus* of 54.6% for the 152 samples. The results demonstrate the presence of *S. aureus* in both cases, regardless of the

situation in the referral hospital. However, the levels of contamination with *S. aureus* were higher in hands of HCW's than mobile phone.

### Antibiotic susceptibility

All 84 *S. aureus* isolates from hands of HCW's and 82 from mobile phone were subjected to antibiotic susceptibility tests. Eleven antimicrobial agents, from different antibiotic classes were used. Some were selected because some studies have shown that large number of bacteria were resistant to them (Akinyemi et al., 2009; Karabay et al., 2007). Antibiotics of human health relevance and availability of antibiotics were also considered. A summary of the percentage of *S. aureus* that were resistant to these antibiotics is provided in Table 2.

A large proportion of the isolates of this study area were resistant to Ampicillin (10 µg) and Penicillin G (10 µg) in both mobile phone and hands of HCW's. There were less resistant groups for CIP, Gen, Dx, CRO, TTC, CAF and Ax antibiotics.

**Table 2.** Details of the *S. aureus* isolates obtained from Hawassa Area, South Ethiopia.

Sample source	No. of sample collected	<i>S. aureus</i> isolates (level of contamination with <i>S. aureus</i> per source)*
Mobile phone	152	82 (53.9%)
Hands of HCWs	152	84 (55.3%)

\*Percentage were calculated from a total of 152 samples studied in both mobile phone and HCWs.

**Table 3.** Antibiotic resistance profiles of *S. aureus* isolated from mobile phone and hands of HCWs.

Sample source	Antibiotic resistance (%)										
	Gen	Dx	CRO	AP	Van	Pen	TTC	Ox	CIP	CAF	Ax
Mobile	14.6	22	15.9	65.9	40.2	47.6	31.7	40.2	31.7	26.6	26.8
Hands of HCWs	11.9	22.6	13.1	71.4	40.5	50.0	33.3	40.5	30.9	23.8	28.6

Percentages were calculated by dividing the number confirmed as *S. aureus* resistant in a particular sample source by the total number of isolated tested.

**Table 4.** The predominant multiple antibiotic resistant phenotypes for *S. aureus* isolated from mobile phone and hands of HCWs obtained from Hawassa Referral Hospital, South Ethiopia.

Phenotype	Mobile phone (n=82)	Hands of HCW's (n=84)
	No. (%)	No. (%)
AP-Pen-Gen	8(9.8)	9(10.7)
AP-Pen-Ox	14(17.1)	13(15.5)
AP-Pen-CRO	5(6.1)	3(3.6)
AP-Pen-Ax	6(7.3)	7(8.3)
AP-Pen-Ax-Dx	3(3.7)	5(5.9)
AP-Pen-Gen-Van	3(3.7)	4(4.8)
AP-CRO-CIP-Van	2(2.4)	4(4.8)
AP-Dx-TTC-Ox-Van	5(6.1)	4(4.8)
Pen-TTC-CIP-CAF-Van	1(1.2)	1(1.2)
AP-Ox-CIP-CAF-Ax-Van	1(1.2)	2(2.4)
Pen-TTC-CIP-CAF-Ax-Van	1(1.2)	0(0.0)
Pen-CAF-CRO-CIP-Ox-Van	1(1.2)	1(1.2)

The percentage representations of the phenotypes were obtained by dividing the number of a particular phenotype by the total number of multiple antibiotic resistant isolates identified in a given area. AP, Ampicillin; Pen, Penicillin; Ox, Oxacilin; CRO, Ceftriaxone; Ax, Amoxicillin; Dx, Doxacilin; Gen, Gentamicin; Van, Vancomycin; TTC, Tetracycline; CIP, Ciprofloxacin; CAF, chloramphenicol.

In general observation, the large percentage of Pen (10 µg), Amp (10 µg), Van and Ox resistant *S. aureus* were isolated from the study area. These were also resistant to other several antibiotics. Therefore, one can easily conclude that these are Methicillin resistant *S. aureus* (MRSA).

#### Multiple antibiotic resistance phenotypes of *S. aureus*

In this study, the multiple antibiotic resistances (MAR) phenotypes were determined for *S. aureus* (Table 3). The

leading MAR phenotypes for *S. aureus* isolated from mobile phone isolates and hands of HCWs were 14 (17.1%) and 13 (15.5%), respectively followed by AP-Pen-Gen which is 8 (9.8%) and 9 (10.7%), respectively. Additionally, MAR phenotypes AP-Pen-Ax were obtained in both mobile phone and hands of HCWs, respectively 6 (7.3%) and 7 (8.3%).

The MAR phenotypes Pen-CAF-CRO-CIP-Ox-Van were 1.2% in both sample sources. However, there is no Pen-TTC-CIP-CAF-Ax-Van MAR phenotypes *S. aureus* in contrast to mobile phone source *S. aureus* which is 1.2% (Table 4). It is thus evident that MAR *S. aureus* was isolated from both sample sources. However, among the

strains from mobile phone were 61.0% and from hands of HCWs were 63.1% isolates developing MAR. Among all MAR phenotypes of *S. aureus*, 20.7% of them were resistance to more than four different antibiotics in mobile phone isolates and 25.0% were from hands of HCW's. In this study, Ampicillin and Penicillin were less effective than other antibiotics. More than 53.6-57.3% of the isolates were resistant to Ampicillin and Penicillin in both cases.

## DISCUSSION

This study described the isolation and antibiotic susceptibility characterization of *S. aureus* from mobile phone and hands of HCW's. The results of this study indicated that 53.4% of the samples were positive for *S. aureus* from mobile phone and 55.3% of the samples were positive for *S. aureus* from hands of HCW's. Several studies have been conducted in different area to evaluate the prevalence of *S. aureus* in mobile phone and hands of HCW's (Mohamad et al., 2010; Kabir et al., 2009; Ulger et al., 2009; Bhat et al., 2011; Rawia et al., 2012; Tagoe et al., 2011; Seuli et al., 2013; Auhim, 2013; Amira, 2010; Yusha et al., 2010; Ilusanya et al., 2012; Jaya et al., 2011). The results reported in this study were higher than other report elsewhere (Auhim, 2013; Bhat et al., 2011; Kabir et al., 2009; Rawia et al., 2012; Tagoe et al., 2011). In contrast to this study, the prevalence of *S. aureus* by Seuli et al. (2013) was 84.0% and Yusha et al. (2010) was 76%. Although, the prevalence of *S. aureus* has been reported to vary with the size and geographic region of the area sampled, a high proportion of these bacteria in mobile phone and hands of HCW's relates to poor hygiene practices.

Based on observations made during the collection of samples, we therefore reported improper hygiene of mobile phone with routine cleaning rate of 5.3% only. About 94.7% of the study participants had never cleaned their mobile phone either daily or weekly which contributed to the presence of *S. aureus* in the mobile phone, especially in those from laboratory and wards. Improving the hygienic conditions of the mobile phone and hands of HCW's after and before performing their procedure in different rooms may reduce the prevalence and transmission of *S. aureus*.

In this study, the prevalence of *S. aureus* in both mobile phone and hands of HCWs were 53.9 and 55.3%, respectively. However, according to the reports of Elkholy and Ewees (2010), the prevalence of *S. aureus* from mobile phone and hands of HCWs were 48 and 31%, respectively. Also, the cleaning rates of their study were similar to the current study. Therefore, it is easy to conclude that less cleaning may contribute to the presence of *S. aureus*.

Also, the antibiotic-resistance profiles of *S. aureus* isolated from the study area is higher than the previous

study (Daka et al., 2012; Brouillette and Malouin, 2005; Petinaki et al., 2001; Moneoang and Bezuidenhout, 2009).

AP (10 µg), PG (10 µg) and Ox (1 µg) were the drugs to which a large proportion of the isolates were resistant in this study as similar to previous study done in this area (Daka et al., 2012) (Table 3). Moreover, only 59.5% of Van (30 µg) were effective against the isolated organisms. This report is also similar to previous study (Daka et al., 2012). Therefore, it is very indicative of the further advanced survey of vancomycin resistance strain of *S. aureus*. Moreover, the assessment of genetic level of the resistance gene is very suggestive according to this study. Also, the resistance pattern of *S. aureus* to Ox (1 µg) is similar with the previous study done in Hawassa Referral Hospital. Hence, it is stress-free to conclude that there is Methicillin resistant *S. aureus* (MRSA) around the study area. High levels of MRSA were reported by Bhat et al. (2011) and Fatma et al (2009) elsewhere from mobile phone and hands of HCW's. Also, high levels of MRSA have been identified in patients in the United States and some European countries (Mark et al., 2003). In these countries, 37.7, 44.4, 34.7, 41.8 and 32.4% of isolates from patients in the Turkey, United States, France, Italy and Spain, respectively, were resistant to Methicillin. These levels, however, are lower than those in our study. Methicillin is rarely used to treat patients in the South Ethiopia. However, Methicillin resistance could be explained by the inter-relationship between beta-lactam resistance and resistance to this drug.

Despite the fact that, the prevalence of the antibiotic resistance pattern was lower in this area as compared to the previous study, still needs great consideration on antibiotic resistance pattern of *S. aureus*. As shown in Table 3, about 65.9% of *S. aureus* were resistant to AP from mobile phone sample and 71.4% of the isolates were resistant to AP. The resistant pattern of the *S. aureus* obtained from hands of HCWs was slightly higher than the isolate obtained from mobile phone. This might be due to an ineffective application of antiseptics such as alcohol on their hand may vary the microenvironment of the organisms. In previous study done in the study area showed that there was no *S. aureus* resistance to CIP (5 µg) (Daka et al., 2012). However, 31.7% of the isolate identified from the mobile phone and 30.9% of the hands of HCWs were resistant to CIP. This might indicate that there is gradual increase of antibiotic resistant pattern at the study area. Therefore, it is better to overcome this problem early.

In MRSA, Methicillin resistance is conferred by the Penicillin binding protein (PBP) 2a that is encoded for by the *mecA* gene (Gündoğan et al., 2005). PBP2a does not readily bind the beta-lactam moiety. However, in MRSA that are exposed to beta-lactam antibiotics, this PBP2a would contribute to the resistance by providing transpeptidase activity to the native PBPs during cell wall synthesis. In our study, the resistant phenotype AP-Pen-

Ox was frequently identified usually with the addition of one or more antibiotics (Table 4). It is thus recommended that future studies should confirm the presence of the *mecA* gene in observed MRSA due to these  $\beta$ -lactamase antibiotics.

Contrarily to our observations, a study reported that larger percentage of *S. aureus* isolate was resistant to CRO, (73.5%); Gen, (47.3%); CAF, (42.1%); CIP, (89.4%) and Ax, 52.6% (Akinyemi et al., 2009). The finding that a large number of *S. aureus* were resistant to PG (10  $\mu$ g), AP (10  $\mu$ g) and Ox (1  $\mu$ g) is, however, a cause for concern and should be further investigated. It is thus our view that the results obtained in this study do not accurately reflect the usage of this antibiotic in the hospital. We cannot explain this phenomenon.

The MAR phenotypes (Table 3) obtained in the study correlated with the percentage of antibiotic resistance. Although the development of resistance to a particular antibiotic depends on the level of exposure to the antimicrobials (Rychlik et al., 2006), there are many other factors that are involved. We are therefore suggesting that molecular methods be used to characterize these isolates for the presence of antibiotic-resistance determinants, which may provide data to support our conclusions.

*S. aureus* is normally resident in humans; therefore the *S. aureus* present in the mobile phone, other medical instrument like stethoscope, thermometers, key boards for computerized equipment and hands of HCW's may have resulted from transmission from humans, patients, workers and children which raises questions regarding the hygiene practices followed.

## Conclusion and recommendation

A large proportion of the isolates obtained were resistant to three or more antibiotics. These were also resistant to Vancomycin (30  $\mu$ g). This was particularly the case in the public setting and is a cause for concern. The high level of MAR *S. aureus* and the implications thereof warrant further investigation. One of the aspects that need to be investigated is the cause of the observed resistance phenotypes. Furthermore, impacts and dynamics of genetic antibiotic determinants should also be investigated using molecular methods.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The author would like to thank the Research Council of College of Medicine and Health Sciences, Hawassa

University, for providing the financial support.

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