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OCCURRENCE OF MULTI-DRUG AND METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) FROM SOURCES OF DOMESTIC WATERS OF GOMBE

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ABSTRACT
Staphylococcus aureus is a major cause of nosocomial and community-acquired infections which is easily transmitted through water/ it is therefore necessary to determine the living susceptibility profile of this species since it can be transmitted from drinking different sources of water. The aim of the present work is to screen different sources of water in Gombe for the occurrence of Staphylococcus aureus with a view to establishing its contribution susceptibility profile for better option on chemotrophic Therefore, water from Tap, Borehole, Stream, Well, Jerry Can vended water, Tank and Pond water which are presently in use for human or animal purpose in Gombe were investigated between the month of March to August . (this period mark the periods of extreme water scarcity due to absence of rainfall to abundance of water from rainfall).Twenty sample each were collected making a total of one hundred and forty (140) samples which were screened for Staphylococcus aureus on Baird parker agar, Mannitol salt agar Cysteine Lactose Electrolyte Deficient agar, and Blood agar. The samples yielded, multi-drugs resistantusing the Kirby Bauer disc diffusion method.against Gentamicin (Potency) ampicillin (Potency), amoxicillin/clavulanate (Potency), Ceftazidime (Potency), Cefuroxime {Potency), Ceftazidime (Potency), Oflaxacin (Potency),Ciprofloxacin (Potency) and Nitrofurantoin {Potency), as decided by CLSI The result showed 30.7% were Multi drug Resistant Staphylococcus aureus, also,Cefoxitin single disc was used to confirm Methicillin Resistance of the samples screened from which 80% of it were confirmed MRSA and the PCR showed the presences of mecA1, mecA2 and PVL genes.
Keywords: Staphylococcus aureus, drinking water, Multi-drugs Resistance, mec A pvl

INTRODUCTION

Staphylococcus was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee join (Ogston A, 1984)). *Staphylococcus aureus* is a Gram-positive bacterium belonging to the family Staphylococaceae and is often found as a commensal on the skin, skin glands and mucous membranes particularly in the nose of healthy individuals (Plata *et al.*, 2009).

Staphylococcus aureus that has developed, through the process of natural selection, acquire resistance to beta-lactam antibiotics, which include the penicillin's (methicillin, dicloxacillin, nafcillin, oxacillin, etc.)). The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *S. aureus* that have no antibiotic resistance, but resistance does make methicillin resistant *Staphylococcus aureus* (MRSA) infection more difficult to treat with standard types of antibiotics and thus more dangerous. In *S aureus,* the emergence of these strains originated from pigs (Habrun *et al, 2011*).

S. aureus and MRSA isolates have been shown to survive river, sea and swimming pool water (Tolba et al, 2008). And thus prompting the hope that this work can isolate MRSA from the most common water bodies in Gombe and environs usually used by both middle and lower class citizens for domestic activities. Molecular identification has been employed so as to make sure this work uses the real MRSA.

Development of antimicrobial resistance by bacteria is now a worldwide health issue, as infection is one of the leading causes of death in the world today. This fact is also as a result of the emergence of multiple antibiotic resistant bacteria known as methicillin resistant *Staphylococcus aureus* (MRSA) with potential of cross resistance to other antibiotics of choice like cefoxitin, oxacillin etc. MRSA is often referred to as a potential killer and one of the tree top superbugs in hospitals multidrug resistant organisms. It is therefore, the aim of the of the work to ascertain the Occurrence of multidrug resistant *Staphylococcus aureus* and

MRSA from different sources of waters in Gombe Township.

MATERIALS AND METHODS

Sample collection the study was undertaken at Gombe township between the periods of March to August 2017. One hundred and forty (140) samples were collected from Tap, Bore hole, Well Jerry Can, Water Tank and Ponds, twenty (20) samples were collated, for each of the variables, labelled, preserved and transported back to the Microbiology laboratory of Gombe state university in brown air tied bottles. For analyses.

Isolation *of S. aureus*

In the laboratory, the water samples were planted by pour plating method on mannitol salt agar, and later on CLED, Baird Parker agar and Blood agar.:On Mannitol salt agar (MSA) (TM Titan Biotech Ltd)which was used to screen for *S. aureus* by observing mannitol utilization confirmed by the resulting medium change to yellow from its initial pink colour) (Cheesbrough 2008.). From the MSA, a colony was picked using a sterile wire loop, and was introduced onto the Baird Parker Agar (BPA) and incubated at 37^{o}/c for 18rs. All colonies that appeared black were later picked and inoculated on Blood agar(TM Titan Biotech Ltd) for Haemolysis (Cappuccino and Sherman 1996), *S. aureus* produced yellow to cream 1–2 mm in diameter colonies after overnight incubation they are *beta* haemolytic colonies are slightly raised and easily emulsified. Cysteine electrolyte deficient (CLED) agar(TM Titan Biotech Ltd) was again introduced with colonies picked from MSA and incubated at 37^{o}/c for 24hrs.Deep yellow colonies produced with total change of the medium colour from green to complete yellow indicates *S aureus*.

The isolates were further subjected to Biochemical tests:

Coagulase test, Catalase and Mannitol utilisation tests. All biochemical test were made in accordance with Cheesbrough 2008. Instruction.

Isolation of MRSA One loopful of the resulting *S.aureus* from the above isolation were picked with wire loop; and was spread over BPA (Biomark Laboratories. Ltd). Supplemented with 6mg cefoxitin for MRSA (MRSA Select TM agar). Resulting black colonies were transferred to blood agar, wherein growth morphology and haemolysis were observed. (Cappuccino and Sherman, 1996 and Cheesbrough 2008). The resulting isolate was preserved on Mueller Hinton agar (MHA) slant ((TM Titan Biotech Ltd)) at 2–8° until needs. (Clinical and Laboratory Standards Institute, 2014.)

Antimicrobial susceptibility testing

All isolates in stock from above isolations were subjected to different antimicrobial agents. Namely, Gentamicin (10ug) ampicillin (10µg), amoxicillin/clavulanate (30µg), Ceftazidime (30ug), Cefuroxime (30µg), Ceftazidime (30µg), Oflaxacin (5µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg), and finally after all this multiple disc, \cefoxitin 30ug was use as single disc. Their susceptibility was tested using the disc diffusion method as standardised by the Clinical and Laboratory Standards Institute (2014).

Molecular analyses: To confirm the identity of the S aureus strains, the extraction of the DNA was done in accordance with the manufacturer's instruction (Instagene Matrix, Biorad®)

Gel electrophoresis

From the DNA extract, 15ul was mixed with a 6x DNA Loading dye for conventional colour tracking in DNA migration. Agarose gel was poured into chamber of PowerPC HC Biorad electrophoresis machine. Comb was removed from the solidified gel and the gel was inserted into the chamber then later 5x buffer was poured. The DNA extract mixture was pipette into the comb holes and later the machine was set at 75^{o}c/40min. This was done to make sure that the DNA extract surely contains DNA.

Primers used

1. Staph756F (5-AACTCTGTTATTAGG GAAGAACA-3)
2. mecA1 (5' - GTA GAA ATG ACT GAA CGT CCG ATA A - 3')
3. mecA2 (5' – CCA ATT CCA CAT TGT TTC GGT CTA A - 3')
4. spa (5' – CGC TGC ACC TAA CGC TAA TG – 3')
5. pvl-F (5' – GCTGGACAAAACTTCTTGGAATAT – 3')

These Primers were used for the amplification of the fragments of the methicillin-resistant gene (mecA) (Perez-Roth *et al.,* 2001)**.**

Positive control used was *S. Aureus (***Staph756F).** Also, negative control was used. By adding DNA of a fungi (Amita *et al,* 2008).

The PCR was done in accordance with procedures instructed by the manufacturer

Comparison of the existence of MRSA

Chi Square was use to see if there is a significant difference in the occurrence of the MRSA samples collected from various sources at P = 0.05

Results

Table 1 Occurrence (%) Rate of *S aureus from Several Water sources in Gombe*

Source	Quantity	Growth
Tap	20	09
Borehole	20	07
Stream	20	17
Well	20	13
Jerry Can	20	14
	20	15
Water Tank		
Pond	20	20
Total/%	140(100)	95(67)

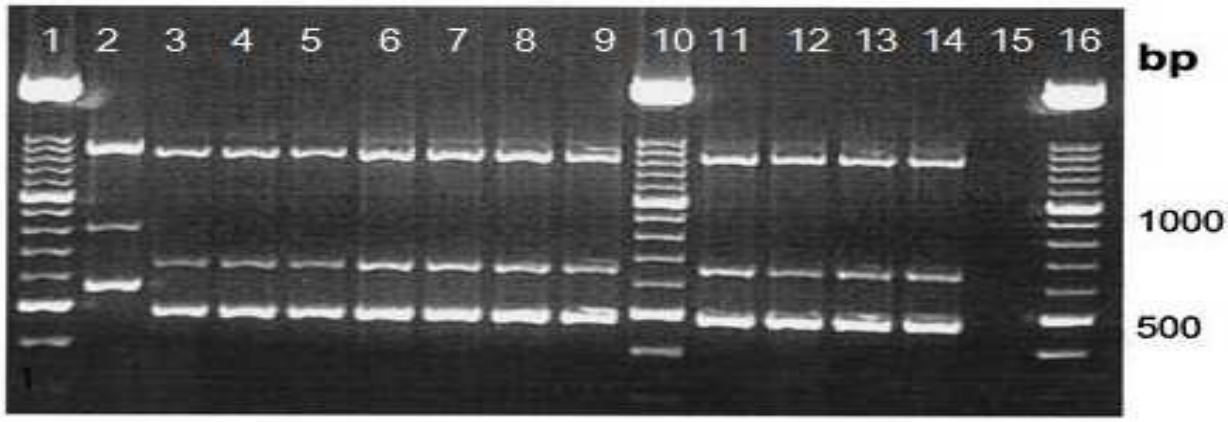
Source	QTY	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP	Inference
Tap	09	R	R	3	R	R	R	08	R	MDRSA
Borehole	07	R	R	R	R	R	R	04	R	MDRSA
Stream	17	R	R	R	R	R	R	15	R	MDRSA
Well	13	R	R	R	R	R	R	03	R	MDRSA
Jerry Can	14	R	R	R	R	R	R	03	R	MDRSA
Water Tank	15	R	R	R	R	R	R	04	R	MDRSA
Pond	20	R	R	R	R	R	R	02	R	MDRSA
Susceptibility (%)		0	0	3(2.1)	0	0	0	39(27.9)	0	70%

Gentamicin (GEN) ampicillin (AMP), amoxicillin/clavulanate (AUG), Ceftazidime (CAZ), Cefuroxime CRX), Ceftazidime (CEF), Oflaxacin (OFL), Ciprofloxacin (CPR), Nitrofurantoin NIT) MDRSA (Multidrug resistant *S. aureus*

- *All 0% _ 100% resistance*

Table 3 Percentage MRSA based on Susceptibility of Isolates to Cefoxitin Disc

Source	Number of Samples	Cefoxitin Resistance
Tap	**09**	**03**
Borehole	**07**	**00**
Stream	**17**	**10**
Well	**13**	**03**
Jerry Can	**14**	**07**
Water Tank	**15**	**02**
Pond	**20**	**18**
Total/%Susceptible	95(100)	**43(30.7)**



1, 10 and Figure1" Picture of gel Documentation for PCR

Position16. were the ladder, 2 positive control. Band 1 was the mecA1, the second mecA2 and the third was the PVL, column 2 to 14 were samples while 15was negative control.

Statistical Comparison of Frequency of MRSA from the Various Water Sources

Chi Square was used to determine the significant difference in the occurrence of the MRSA samples collected .After computation the result shows that there is no significant difference in frequency of occurrence of MRSA among the water sampled. Between the samples isolated in relation to the environment it occurs. I.e. all samples collected from different sources have equal chance of producing MRSA.

DISCUSSION

The fact that *Staphylococcus aureus* is known to be isolated from different parts of the environments is well established (Percival *et al*., 2004). In the present study, 67% of water samples collectively yielded *staphylococci*, 32.9% of these isolates confirmed as *S. aureus* through coagulase test (Table 2) and 30.7 were MRSA. Higher percentages of *S. aureus* occurrence in water were reported worldwide (Harakeh *et al.,* 2006; Faria *et al*., 2009), also in Makkah, Saudi Arabia (Mihdhdir, 2009; Abulreesh and organji, 2011). There are many reasons for potential concern when *S. aureus* are present in drinking water supplies. (Allen et al., 2004), Common food preparation practices such as washing boiled potatoes, pasta, shellfish, and cooling of boiled eggs could possibly leave these food items contaminated with *S. aureus*. Likewise, if the food items used for preparation of salads are left at room temperature, or improperly refrigerated, the possibility of staphylococcal food intoxication certainly existsIt was observed in this study that most of the *S. aureus* tested in this study showed multiple resistances to antibiotics. This observation is similar to a study carried out in Nepal on sewage treatment plant (Rajbanshi, 2008). It has been reported that microbial resistance to antimicrobials and heavy metals.

This finding agreed with previous research which reported the isolation of pathogenic microorganisms including *S. aureus* from effluent of soil, water and abattoir (Ogbonna, 2014). In case of antibiotic resistance, the result showed that all the isolates were resistant to ceftazidime (10 µg), this is similar to the report of (Al-Sa'ady *et al*. 2014) in which all the strains of *Staphylococcus epidermidis* were observed to be resistant to the same antibiotic but the concentration used was not indicated.

This study have also observed that 30.2% of the isolates were resistant to Cefoxitin which is greater than the 24.0% reported from wastewater that originated from slaughter houses and municipal sources in Germany by Bohn *et al (2014)*. This may be due to the change in geographical location of the study areas

The multidrug resistant hospital-acquired MRSA (HA-MRSA) strains and of *S.aureus* and their intrinsic resistance to beta-lactam antibiotics confer limited treatment options to the most available and less costly antibiotics in developing countries as it was reported by Vladimir *et al*,(2011). And this is a serious issue considering the recession situation of present Nigeria

The susceptibility and resistance pattern of the isolated S. *aureus* revealed a high level of resistance amongst the *S. aureus* to all the commonly used antimicrobials, Gentamicin, ampicillin, amoxicillin/clavulanate, Ceftazidime. Cefuroxime, Ceftazidime, Oflaxacin, Ciprofloxacin, Nitrofurantoin, but, 27.9% of the isolates were susceptible to Nitrofurantoin. The results were comparable to those in a previous study carried out by Nwankwo and Nasiru, (2011).and the resistance profile in this work appeared higher this may be due to the fact that the isolates were collected from water and may have originated from human other animals bodies which were washed down into water bodies and for the isolates to survived different environments they have to acquire more phenotypic feature.

In the present study, *Staphylococcus aureus* susceptibility and resistance seems not to conform to usual norm of having other non-Betalactams as the most active drug. The MRSA showed a high level of resistance to all antimicrobials in general. Also most of the MRSA in this study were actually resistant to many classes of antimicrobials at the same time and thus qualify as multiply drug resistant *Staphylococcus aureus* (MDR-MRSA), which is in agreement with the work of (Kesah 2003) on Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta.

The MRSA mechanism of resistance is believed to be due to the presence of the *mecA* gene. Whose genotypes 1 and 2 as well as PVL were detected among 30.7% of the encountered Isolates. This induces a thicker cell wall with a decreased production of PBPs (Oliveira and de Lencastre, 2002), thus can felicitate spread of epidemic strain in debilitated patients. The methicillin-resistant phenotype can be highly heterogeneous, making it difficult to detect by conventional anti-microbial susceptibility test methods, hence necessitate the use of PCR incorporated mec A detection protocols.

Conclusively, the study established that multidrug and methicillin resistant *Staphylococcus aureus* are common in the various domestic sources of water in Gombe

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