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Susceptibility pattern of *Staphylococcus aureus* isolated from cows in Obiaruku Delta State
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Abstract

Nasal carriage of *Staphylococcus aureus* is a well-known factor for subsequent infection and transmission. This study accessed the nasal carriage of *S. aureus* live stocks and the sensitivity profile of isolates to antibiotics. Nasal swabs were collected from hundred (100) healthy cows. The identification of *Staphylococcus aureus* was performed in accordance with the standard laboratory protocol including typical colonial morphology, gram staining, catalase, coagulase hydrogen sulphide test. Also, antibiotic susceptibility testing of the isolates was carried out using the Kirby Baur method of disc diffusion testing. From the results obtained. The morphology and biochemical tests of samples isolated from nostrils of 100 healthy live stocks (cow) revealed that a total of 34 (68%) of the isolates were identified as *Staphylococcus aureus* out of the hundred (100) samples. Antibiotic sensitivity pattern of the *Staphylococcus aureus*, recorded that the majority of the bacterial isolates were resistant to the commonly used antibiotics which including ampiclox and erythromycin. It was equally observed the isolates were slightly resistant to the ciprofloxacin, ceftriaxone, and streptomycin. Ceftriaxone recorded the highest level of susceptibility followed by streptomycin and ciprofloxacin which has the same percentage of sensitivity. Erythromycin, and ampiclox recorded the least susceptibility. Sensitivity test result obtained showed that erythromycin has the highest number of resistances, and ceftriaxone had the least degree of resistance. The result obtained, therefore indicates the importance of investigating *Staphylococcus aureus*

colonization of nasal mucosa in order to ascertain the level of antibiotic susceptibility of *S. aureus* infection and thus develop intervention strategy as well as treatment possibilities.

Keywords: Cow, antibiotics, Obiaruku, susceptibility, *Staphylococcus aureus*.

Introduction

Ever since the introduction of antimicrobial drugs that are β -lactamase-stable into medical use, methicillin-resistant *Staphylococcus aureus* (MRSA) have surfaced universally as significant nosocomial pathogens; even their prevalence in the community is rising considerably. Though *S. aureus* has been discovered to be one of the commonest aetiology of mastitis in bovine and even some other serious diseases of animal such as septicaemia, wound, joint infections, bone, and MRSA strains infrequently have been isolated from animals. Strains of MRSA have now been isolated from cows with mastitis, dogs and horses with lesions, and cats and dogs that were carriers (Aires-de-Sousa *et al.*, 2006). The transmission of MRSA between animals and the human beings (e.g., dogs, pigs, horses,) has been reported (Van Cleef *et al.*, 2016), nevertheless the transmission between humans and cows has not, to our knowledge. We explain a first recognized case of MRSA transmission between a person and cows.

Staphylococcus aureus usually colonise the anterior nares of almost all the animals that are domesticated and 30% of humans (Wardyn *et al.*, 2015). Livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) usually pose a zoonotic

risk, mostly for the people working in nearness to livestock (Dahms *et al.*, 2014). The greatest livestock concentrations in European Economic Area (EU/EEA) European Union countries in 2013 were in Benelux countries (Belgium, the Netherlands, and Luxembourg), Nordic countries (Denmark and Norway) and Mediterranean islands (Cyprus and Malta) (Eurostat *et al.*, 2016). Several lineages of LA-MRSA have been described (Chuang *et al.*, 2015). The most widespread LA-MRSA lineage in the North America and Europe is regarded as clonal complex (CC) 398 which include multilocus sequence type (MLST) ST398, that is known to be regularly associated with the swine, however it has as well been identified in poultry and cattle. The MRSA CC398 carriage is frequent in persons with regular contact with livestock, including swine farmers and individuals living in environments with elevated livestock density. The effect of this carriage on otherwise healthy people seems be low and LA-MRSA infections usually possess a related viciousness to other MRSA infections (CunyCKöck and Witte, 2013).

The death of four persons in 2014 resulting from LA-MRSA CC398 in Denmark caused substantial media and political awareness in Nordic countries and the European Parliament concerning the potential burden of LA-MRSA in pigs and humans (Statens *et al.*, 2014). The management risk of options for the LA-MRSA involves the detection of method of transmission chains to stop the process of transmission. Furthermore, constant wearing of face masks during work in stables of pig has been known to reduce the carriage of MRSA rates by 37% (Van *et al.*, 2014).

A survey in 2007, that obtained data on the MRSA and the LA-MRSA, which include the screening of samples from 21 staphylococcal reference laboratories in 15 countries. It has been have reported in eight countries where LA-MRSA cases has been isolated from humans, which include clinical isolates; above 2% proportion of MRSA that were LA-MRSA in about four countries and in one region in Germany (Van *et al.*, 2011).

Staphylococcus aureus can result to nosocomial infections. The seriousness of infections caused by MRSA contributes to the economic weight of patient owing to longer period of stay in hospital. *Staphylococcus aureus* is a microorganism that normally colonise the skin and the mucosal surface of healthy persons, however it is known to be one of the commonest causes of hospital and community acquired infections. These bacteria are capable of causing infections to every organs and tissues beginning from surface skin infections to severe, life threatening infections mostly in immunocompromised and hospitalized patient (Wertherim *et al.*, 2005). The aims of the present study were to determine the frequency of nasal carriage of *S. aureus* among healthy ruminants and to investigate the antibiotic susceptibility of pattern *Staphylococcus aureus* among cows being slaughtered in Obiaruku, Delta State, Nigeria.

Methods

Specimen Collection

After thorough explanation to herders, one hundred (100) nasal samples were collected from healthy cows from the slaughter in Obiaruku, Delta State using sterilized swab sticks by inserting and rotating one swab tip in both nares for about 5 seconds. The obtained samples were processed immediately in the Department of Pharmaceutical Microbiology Delta State University Abraka, Nigeria.

Culturing of Sample and purification:

The swab sticks containing samples were swirled into different sterile nutrient broth and then incubated for 24 hours at 37°C. Sequel to the incubation, bacterial isolates were then purified by the process of inoculating them in an appropriate bacteriological medium, mannitol salt agar (MS Agar). The bacteria with the characteristic features on these media were carefully and aseptically isolated and be made subject to microscopic and appropriate biochemical tests for ideal identification.

Identification and confirmation of Isolates:

The several isolates gotten were then characterized by subjecting these isolates to biochemical and morphological tests. The bacterial isolates were gram stained and some

biochemical tests, such as Catalase test. Coagulase test were conducted to further identify the microorganisms to species level (Cheesbrough, 2004).

Antimicrobial screening/sensitivity studies:

Antimicrobial susceptibility profile of these bacterial isolates was assessed using the Kirby-Bauer disk diffusion procedure following the guidelines of Clinical Laboratory Standard Institute (CLSI) 2005 as explained by (Akbar and Anal, 2013). Antibiotic discs (Oxoid, UK) which contain the following antibiotics were used: Ciprofloxacin (10µg), Streptomycin (30µg), Erythromycin (15µg), Ceftriaxone and Ampiclox (10µg). Individual antibiotic disc was carefully dropped on the agar plate and incubated at 37°C for 24hours. The clear zone surrounding

each antibiotic disc was observed, measured in millimetres and then recorded.

Results

The morphology and biochemical tests of samples isolated from nostrils of 100 healthy live stocks (cow) were carried out. Total of 34 (68%) of the isolates were identified as *Staphylococcus aureus* out of the hundred (100) samples collected as shown in Table1. Antibiotic sensitivity pattern of the *Staphylococcus aureus* as presented in Table 2. Majority of the bacterial isolates were resistant to the commonly used antibiotics which include ampiclox and erythromycin as shown table 3. Ceftriaxone recorded the highest level of susceptibility followed by streptomycin and ciprofloxacin which has the same percentage of sensitivity.

Table 1: Incidence of *Staphylococcus aureus*

Study population	Number Positive	Percentage (%)
100	35	68

Table 2: Antibiotic Susceptibility pattern of *S. aureus* Isolates

Antibiotic	Number Sensitive	% Resistance
Erythromycin	11	11
Streptomycin	29	29
Ampiclox	7	7
Ciprofloxacin	29	29
Ceftriaxone	32	32

Table 3: Antibiotic Susceptibility pattern *S. aureus* Isolates

Antibiotic	Number Resistant	% Resistance
Erythromycin	23	23
Streptomycin	5	5
Ampiclox	27	27
Ciprofloxacin	5	5
Rocephin	2	2

Discussion

This present study pointed out a relatively elevated incidence of *S. aureus* nasal carriage (68%) in healthy cattle. This finding is consistent with previous report by (Eva *et al.*, 2007). This finding disagrees with earlier reports gotten from Norway and Sweden which evaluated possible sources of *S. aureus* in the dairy herds (Mork *et al.*, 2012). The colonization of the ruminant's nasal cavities with *S. aureus* could be regarded as a likely source of the infections of staphylococcal origin (Vautor *et al.*, 2004). The incidence detected in this present study was, though, more than the 43.7% that was reported for the similar species in Saudi Arabia (Alzohairy *et al.*, 2011).

Staphylococcus aureus usually colonize the anterior nares of almost all the domesticated animals and the nostrils of about 30% of humans. It has been recognized that cows have become one of the key supplies of transmission for several pathogenic including *Staphylococcus aureus* (Jaffe *et al.*, 2000). *Staphylococcus aureus* is normally a commensal and even a chief opportunistic pathogen which is known to cause a large variety of diseases in both animals and humans, with an elevated impact on the public health even at the livestock industry (Luzzago *et al.*, 2014). This organism has been discovered globally as the main pathogen that causes mastitis in sheep, lactating cows and goats (Vautor *et al.*, 2009). Though the udder has been considered to be the major source of infections, but other regions which include nares are possible contamination sources for the udder and milk in the dairy farms (Vautor *et al.*, 2009). In recent times, the isolation of *S. aureus* from nares of animals from farm has frequently been reported (Nemeghaire *et al.*, 2014). The rising prevalence of *S. aureus* found in the nasal cavities of small ruminants could be one of the predisposing factors for the following Intra-Mammary Infections (IMI) that needs extra relative studies. On this note, the small ruminant's nasal cavity has been anticipated to be the of *S. aureus* primary reservoir by certain researchers (Mork *et al.*, 2012).

Antibiotic sensitivity pattern of the *Staphylococcus aureus* revealed that the

majority of the bacterial isolates were resistant to the commonly used antibiotics which include ampiclox and erythromycin. It was equally observed the isolates were slightly resistant to the ciprofloxacin, ceftriaxone, and streptomycin. This finding is consistent with previous reports (Aires-de-Sousa *et al.*, 2006) where it was known that though *S. aureus* has been discovered to be one of the most common cause of mastitis in bovine and other severe animal diseases which include joint infections, bone, septicemia, wound, and strains of MRSA have been isolated occasionally from animals. Surfacing of organisms that are drug resistant, most importantly MRSA is of a great concern to both public health and animal health. There is growing evidence that *S. aureus* could colonize and even cause infections in companion animals even animals within the food chain (Petinaki *et al.*, 2012). Moreover, studies have documented a lower *S. aureus* prevalence in bovine as well as bovine mastitis (Fluit 2012). Similarly, obtained results of another study (Bagcigil *et al.*, 2007) indicated that MRSA was not isolated from any of the cattle nasal swabs. However, this finding was observed to be inconsistent with the findings of Spohr *et al.* (2011). As zoonotic transfer of MRSA has been reviewed between different animals and humans (Weese *et al.*, 2010), more research should be done in this field in different parts of world to enable us apply necessary management and health measures to prevent the spread of MRSA strains which have now been isolated from cows with mastitis, dogs and horses with lesions, and cats and dogs.

It was observed that ceftriaxone recorded the highest level of susceptibility followed by streptomycin and ciprofloxacin which has the same percentage of sensitivity. Erythromycin, and ampiclox recorded the least sensitivity. This is similar to what was reported by (HumansÉva *et al.*, 2007). Also, from the study, *Staphylococcus aureus* showed a high resistance to erythromycin and the penicillin (ampiclox). This may be attributed to the fact that, on the farm, clinical cases of mastitis were treated with intramammary infusions which contain cephalosporins or penicillin, aminopenicillins. Individual cow received drying-off therapy with cephalosporins or cloxacillin. The antimicrobial

drugs use could have resulted to the appearance of MRSA in the dairy farms (Humans Éva *et al.*, 2007). The widespread use of β -lactam antibiotics for animal treatment in Iran may explain the emergence of penicillin resistance within the microbial strains that are isolated from farms animals in numerous reports from Iran (Jamali *et al.*, 2014). These results point out the need for efficient control of transmission of penicillin resistance among ruminants.

The antimicrobial resistance profile of *S. aureus* has become of great concern because β -lactams, tetracyclines and macrolides are the commonly available antibiotics used for the staphylococcal infection's treatment and the level of resistance to these antibiotics is increasing rapidly amongst the staphylococci (Fluit *et al.*, 2001).

Several evidences have been provided by many studies concerning the transmission of antibiotic-resistant *S. aureus* (especially Methicillin-Resistant *Staphylococcus aureus* (MRSA) amongst animals, farmers and farm environments (Loncaric *et al.*, 2013). In addition, the transfer of antibiotic-resistant *Staphylococcus aureus* or their resistance genes to humans via the food chain, dairy products and meat (Normanno *et al.*, 2006), has before now been reported. This can signify a zoonotic risk factor for colonization of human or hard-to-treat infections. So, extra awareness needs to be created towards the antibiotic-resistant *S. aureus* detection in order to attain a successful treatment for infections caused by *S. aureus*. *Staphylococcus aureus* nasal carriage in human medicine has been comprehensively studied in healthy individuals and patients (Olalekan *et al.*, 2014).

Generally, the antibiotic resistance patterns noticed for isolated *S. aureus* from the cattle nasal cavity seems to mirror the drug therapy patterns in these species of animal. This was further confirmed by the veterinarians within these studied regions where treatment plan of ruminant infections is solely based on use of around 30% tetracycline, 50% penicillin and 20% of some other antibiotics which include gentamicin and tylosin and the not often used macrolide antibiotics. Presence of *S. aureus*

nasal isolates that are resistant to antibiotic from the cattle in this study points out that these species could be the cause of human infections. The transmission of *S. aureus* and its methicillin resistant variant between animals and humans has been commonly reported through direct contact (Deiters *et al.*, 2015) or indirect routes such as the environment and food chain (Verkade *et al.*, 2014).

In conclusion, these findings show that the nares of healthy cattles may represent a good reservoir for antibiotic resistant *S. aureus*, underscoring the need for further extensive research to work out appropriate control and prevention measures. To my knowledge, this is the first survey in Obiaruku investigative study on the prevalence and resistance characteristics of *S. aureus* nasal isolates from cattle.

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