ORIGINAL RESEARCH

Prevalence and Susceptibility of Animal-associated *Staphylococcal* Species Isolated from the Urine of Healthy Students in Jos, Nigeria

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ABSTRACT

Background: Globally, about 1,000,000 cases of illness and millions of deaths occur every year due to zoonoses. Human colonization and infections with livestock associated *Staphylococcal* species has been reported mainly among farm workers.
Objective: The purpose of the study was to establish the profile of *Staphylococcal* species in healthy community carriers.
Methods: Urine samples obtained from 217 healthy volunteers were screened for staphylococcal species and isolates characterized by conventional cultural and biochemical methods including rapid test kits (Microgen ID test kit). The isolates were subjected to susceptibility tests against a panel of antibiotics.
Results: Out of 217 samples collected; 171 isolates were positive for staphylococcal species giving a prevalence of 78.8 %. With the Microgen ID® test kit, 73 (33.6 %) isolates were identified as *S. aureus* and 62 (28.6 %) isolates were non-*Staph aureus,* some of which are associated with animals. The staphylococcal species identified are *S. hominis* (humans), *S. hyicus* (swine, cattle, poultry) *S. intermedius* (carnivores, domestic horses, poultry), *S. xylosus* (human skin, rodents, mammals, birds), *S. schleiferi* (human clinical specimens, carnivores), *S. cohnii* (humans) and *S. haemolyticus* (human skin, primates, horses, artiodactyls). Only *S. cohnii* (18.1 – 81.8 %) and *S. intermedius* (12.5 – 56.3 %) were susceptible to all test antibiotics.
Conclusion: The detection of animal-associated organisms isolated from the urine of healthy human volunteers and their susceptibility pattern is of public health concern. There is a need to properly identify the organisms in specimens and select the appropriate antibiotic for treatment.

Keywords: *Staphylococcal* species; Urine; Healthy volunteers; Livestock; Zoonoses; Students

INTRODUCTION

Many pathogens such as bacteria, viruses, parasites, and fungi could infect both animals and humans. As a result, diseases caused by the pathogens may be transmitted between humans and animals. These diseases (Figure 1) are collectively known as ‘zoonotic’

diseases and make up more than 60 % of all known human infectious diseases and up to 75% of new or emerging infectious disease are of zoonotic origin[1]. These infections may be classified as endemic zoonoses which are present in many places and affect many people and animals; epidemic zoonoses which are sporadic in temporal and spatial distribution; and emerging and re-emerging zoonoses which are newly appearing in a population or have existed previously but are rapidly increasing in incidence or geographical range[2]. The re-emerging zoonoses are exemplified by viral diseases such as pandemic influenza H1N1 2009, yellow fever, avian influenza (H5N1) and (H7N9), West Nile virus and, the Middle East respiratory syndrome coronavirus (MERS-CoV)[2]. The reported high carriage rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among people in contact with pigs indicates that livestock may serve as an important source of community acquired MRSA (CA-MRSA)[3-7]. As most of the emerging zoonotic infections have reservoirs in animals or vectors, occurrence of such diseases in humans often cannot be precisely predicted. Therefore, investigation at the first sign of emergence of a new disease in animals that has the potential to jump species barrier is important for early detection of disease threats from zoonoses[2].

A previous study on the prevalence and urine carriage of MRSA in healthy volunteers sampled from a student population revealed the presence of MRSA and other *Staphylococcus* species associated with animal origin[8]. The non-aureus *Staphylococcus* species include *S. hominis*, *S. hyicus*, *S. intermedius*, *S. xylosus*, *S. schleiferi*, *S. cohnii* and *S. haemolyticus*. These listed non-*Staphylococcus aureus* species are normally associated with animals such as pigs, cattle, dogs and birds except *S. hominis* and *S. haemolyticus* which are found in humans. Consequently, it is intriguing to have found these otherwise animal associated *Staphylococcus* species in urine samples collected from healthy volunteers. Even more of a concern is that the volunteers are student population and not farmers. In addition, most of them live within the University environment and not near animal farms. This poses a concern as to where the students could have contacted these species. Indeed, it is a concern due to the pathogenicity of these species[9-14]. Consequently, this study was undertaken to establish the profile of the animal associated *Staphylococcal* species isolated from the healthy volunteers.

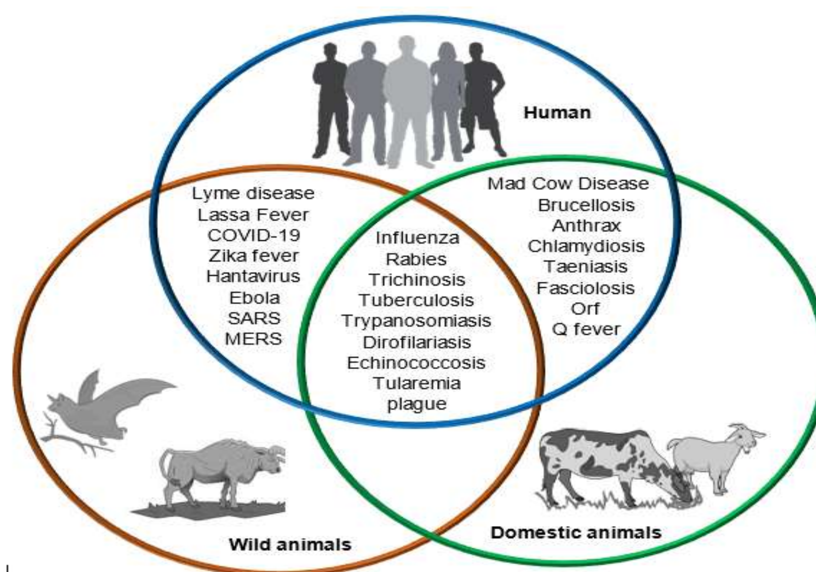

Figure 1: Schematic illustration of some zoonotic diseases and their affected populations.

METHODS

Sample Collection

Urine samples were collected from healthy students at the University of Jos who were not on any antibiotics (or previously taken any antibiotics) three to four weeks before samples were collected from adult male and females of average age 25 years. Mid-stream urine were collected aseptically from healthy volunteers into sterile universal bottles. Convenience sampling method was used to target 200 to 250 volunteers. The urine samples collected from 217 adults were analysed within four hours (4 hrs). Informed consents were obtained from the volunteers after detailed explanation which buttressed on the fact that it is the by-product (urine) that is being requested for and the data will not be traced to any of them. The informed consent form was structured to assure anonymity.

Isolation of organisms

The urine samples were inoculated into sterile nutrient broth and incubated for 18-24 hours at 37°C. Streaks were made from the overnight cultures on to dried sterile mannitol salt agar (MSA) and incubated at 37°C for 24 - 48 hours. Characteristic colonies were purified by re-streaking on mannitol salt agar and incubated. Isolated characteristic colonies were inoculated into nutrient broth and nutrient agar slants, incubated at 37°C overnight and kept for further characterization to establish their identity.

Characterisation and identification of Staphylococcal isolates

Isolates were characterised using established microbiological methods, which included colonial morphology, Gram's stain reaction, catalase and coagulase tests. Isolates that were Gram-positive cocci, catalase positive and coagulase positive were considered as *S. aureus*. Microgen™ STAPH-ID kit was used for the final identification of the isolates. The Microgen Kit is a single microwell test strip which employs 12 standardised biochemical substrates to identify the genus *Staphylococcus* on the basis of ample computer analysis of published databases. Single colonies of 24-hour cultures of the coagulase positive isolates were emulsified in the suspending medium of the Microgen test kit and mixed thoroughly. With the aid of sterile Pasteur pipette, 3 drops of the bacterial suspension were added to each well of the strips. Wells 10 and 11 were overlaid with 3 drops of mineral oil. The top of the microwell test strips were then sealed with adhesive tape and incubated at 37 °C. The test strips were read after 24 hours against a template. The readings were fed into a software (Microgen ID) and the isolates were identified to sub-specie level.

Susceptibility of *Staphylococcal* isolates to the test antibiotics

The antibiotic susceptibility patterns of the staphylococcal isolates were determined using the disc diffusion test method of the Clinical Laboratory Standards Institute formerly (NCCLS)[15]. Sterile 20 mL aliquots of Mueller-Hinton agar were poured into sterile Petri dishes and allowed to set. Standardised (0.5 McFarland turbidity standard) overnight cultures of the isolates were used to flood the Mueller-Hinton agar plates. The excess inoculum was poured out and the plates were dried in the incubator for 10 minutes. Standard antibiotic discs were aseptically applied to the plates ensuring that every part of the discs touched the agar and was left at room temperature for one hour before incubating in an inverted position at 37 ºC for 18-24 hours. The test antibiotics include penicillin (1.5 IU), cefotaxime (30 µg), oxacillin (1 µg), vancomycin (30 µg), ofloxacin (5 µg), ciprofloxacin (30 µg), co-trimoxazole (25 µg), erythromycin (15 µg). tetracycline (10 µg), cefoxitin (30 µg) and gentamycin (10 µg). The inhibition zone diameters were measured to the nearest millimetres using a transparent plastic rule and classified accordingly as sensitive, intermediate, or resistant based on the CLSI interpretive chart for zone sizes[15]. Three determinations were done for each isolate and the average of the readings calculated.

RESULTS

Characterisation and identification of Staphylococcal isolates

The prevalence of *Staphylococcal* isolates is as shown in Figure 2. From the total urine samples collected (217) and cultured on Mannitol Salt Agar (MSA), 171 isolates were categorized as staphylococcal species, out of which 135 (62.2 %) were coagulase positive. Staphylococcal isolates identified with culture methods (135) were further tested with the Microgen™ STAPH-ID kit; 73 (33.6 %) of these were confirmed as *S. aureus* (Figure 2) and 62 (28.6 %) were non-*Staph aureus*. Forty-six (21.2%) of the total samples collected did not show any growth on MSA..

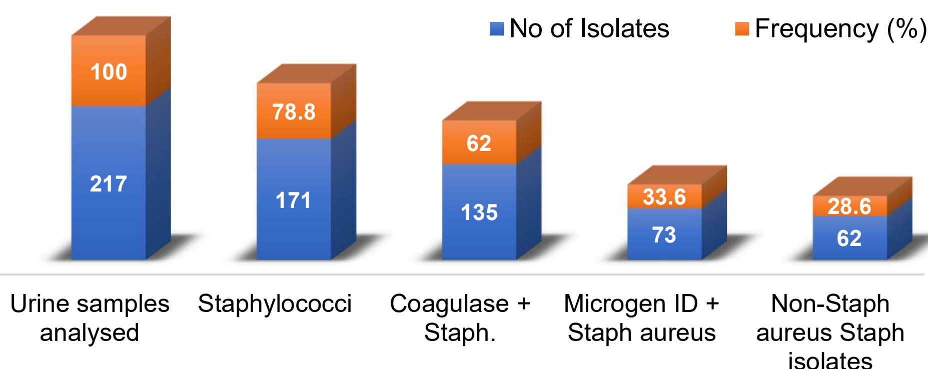

Figure 2: Proportion of samples positive for *Staphylococcus* species

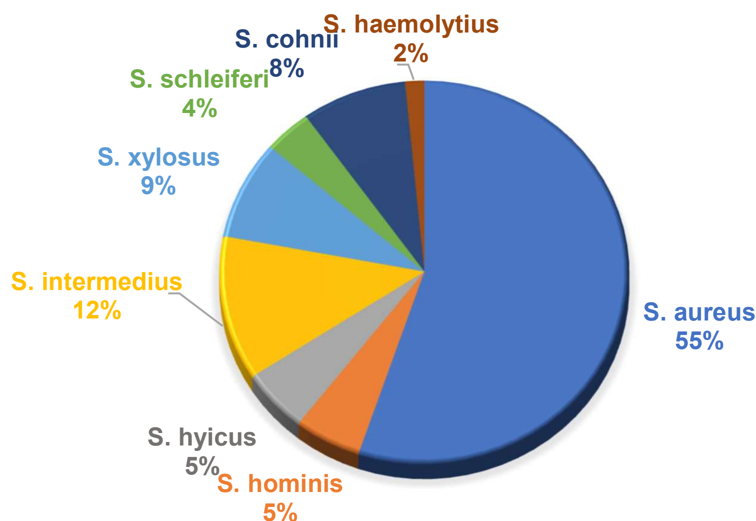

Figure 3: A chart showing distribution of coagulase positive *Staphylococcal* species (n=135)

The percentages of staphylococcal species, both *S. aureus* and non-aureus species present in the urine samples are shown in Figure 3. The percentages are based on 135 coagulase positive isolates. Non-*Staph aureus* were identified as *S. cohnii* (8.1%), *S. haemolyticus* (1.5%), *S. hominis* (5.2%), *S. hyicus* (5.2%), *S. intermedius* (11.9%), *S. schleiferi* (3.7%), *S. xylosus* (8.1%) and *S. lugdunensis* (0.1%).

Susceptibility of *Staphylococcal* isolates to the test antibiotics

The susceptibility of the isolates is as shown in Figures 4. Out of 11, *S. cohnii* isolates, nine (9) were susceptible to erythromycin, ciprofloxacin, cotrimoxazole and vancomycin. The beta-lactams generally were less effective. One of the two isolates of *S. haemolyticus* showed susceptibility to the test antibiotics except tetracycline, cefoxitin, penicillin and ofloxacin. The only isolate of *S. lugdunensis* was resistant to all the antibiotics tested except erythromycin and vancomycin. Ofloxacin and penicillin were the least effective against most of the isolates. The absence of bars in Figure 4 indicates resistance of the organism(s) to the test antibiotic(s). *S. schleiferi,* displayed only 20% or less susceptibility to the test antibiotics. Only *S. cohnii* (18.1 – 81.8 %) and *S. intermedius* (12.5 – 56.3 %) were susceptible to all test antibiotics.

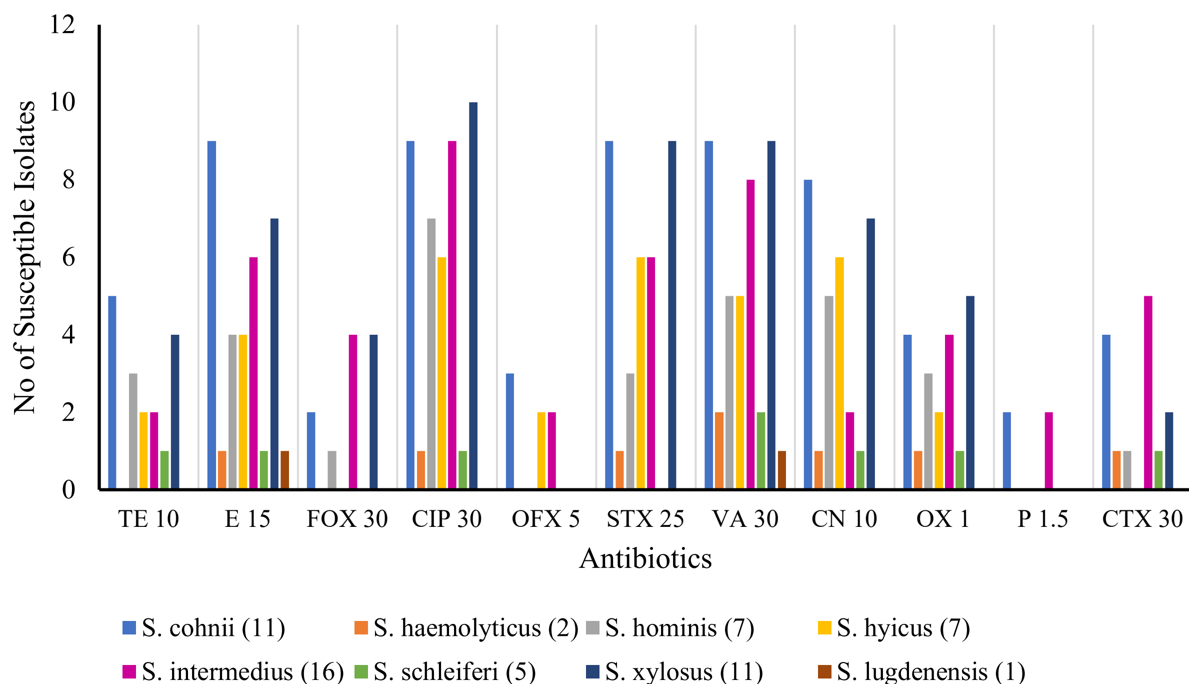

Figure 4: Susceptibility of animal-associated *Staphylococcal* isolates to test antibiotics

Key: P – Penicillin; CTX – Cefotaxime; OX – Oxacillin; CN – Gentamicin; VA – Vancomycin; STX – Co-trimoxazole; OFX – Ofloxacin; CIP – Ciprofloxacin; FOX – Cefoxitin; E – Erythromycin; TE – Tetracycline.

DISCUSSION

Characterisation and identification of Staphylococcal isolates

The conventional cultural method of *Staphylococcus aureus* identification gave a higher positive value for the prevalence. However, subjecting them to Microgen ID test kit, showed that some of the isolates identified as *Staphylococcus aureus* by the cultural method were other staphylococcal species such as *S. hominis, S. hyicus, S. intermedius, S. xylosus, S. schleiferi, S. cohnii and S. haemolyticus*. A substantial proportion of the isolates were inaccurately identified by the conventional method. These non-*Staph aureus* species were normally associated with animals such as pigs, cattle, dogs and birds except *S. hominis* and *S. haemolyticus* which are also found in humans[16]. It is a concern to find these otherwise animal associated *Staphylococcus* species in urine samples collected from healthy volunteers. Even more of a concern is that the volunteers are student

population and not farmers. In addition, most of them live within the University environment and not near animal farms. However, a possible explanation for the presence of these animal-associated *Staph.* species in healthy humans may have resulted from previous exposure to pets or farm animals. It is usual to find herds of cattle in residential areas in Nigeria. The student hostels are frequently visited by cattle led by herders, dogs, cats, and birds of all kinds. While this study may have been about students, pet owners, farmers and veterinarians are at risk of zoonotic transmission.

The routine biochemical tests may misidentify the animal Staphylococcal species as *Staph aureus*. Microgen ID test is a more specific method of identifying Staphylococcal species. As a result, more specific tests such as Microgen ID test and other tests and analyses should be used to ascertain the specific *Staph*. species. Other tests utilized in identifying *S. aureus*, include production of protein A, cell-bound clumping factor, extracellular coagulase, and heat-stable nuclease in addition to molecular methods[17]. However, in this study, Microgen ID test kit was used, and the results showed that some of the isolates identified as *S. aureus* by culture methods were actually other staphylococcal species. Careful identification of these animal staphylococcus species in diagnostic laboratories cannot be over emphasized and these organisms should be considered pathogenic until proven otherwise. They should no longer be labelled as contaminants and non-virulent.

Some studies have shown that these staphylococcal species have been implicated in human diseases. *S. schleiferi* are known to cause wound infection, prosthetic infections and bacteremia, however, it was the causative agent for meningitis in a 6-year-old girl[11]. *S. cohnii* is another opportunistic pathogen which has been implicated in brain abscesses, skin and soft tissue infections, acute cholecystitis, brain abscess, endocarditis, pneumonia, urinary tract infection and septic arthritis and other systemic human infections[14,18]. *S. haemolyticus* may be responsible for septicaemia, peritonitis, otitis, native valve endocarditis, wound, bone, joint and urinary tract infections[19, 20]. *S. xylosus* is a cause of urinary tract infection mainly in young women and it is also known to cause infective endocarditis[10]. *S. hyicus*, is found on the commensal flora of animals and is a causative agent of exudative epidermitis[21]. *S. intermedius* causes otitis externa, pyoderma and wound infections in pets and while they are rare in humans, there have been reports of fatal human infections[9]. *S. lugdunensis* has been implicated in central nervous system infections, endocarditis, endophthalmitis, osteomyelitis, peritonitis, prosthetic joint infections, urinary tract infections, and systemic infections[22].

Susceptibility of *Staphylococcal* isolates to the test antibiotics

The isolates showed varying levels of susceptibility to the test antibiotics. The penicillins were the least active. The β-lactams were not as effective against the organism compared to ciprofloxacin, erythromycin, vancomycin and cotrimoxazole. Cefotaxime is a 3rd generation β-lactam and is supposed to be active against isolates that are resistant to the 1st and 2nd generation antibiotics. That some of the organisms are resistant to cefotaxime is a concern as the choice of antibiotics to treat resistant isolates will be limited.

It is interesting that the old antibiotics such as erythromycin and co-trimoxazole which presently are not commonly prescribed showed good activity. The activity of co-trimoxazole was better than that of cefotaxime a 3rd generation cephalosporin but comparable to those of ciprofloxacin and vancomycin. The performance of co-trimoxazole may be due its limited prescription in veterinary practice. In addition, years back, co-trimoxazole was commonly prescribed in human practice but with the advent of high-level resistance, newer antibiotics were used instead. It is perceived that the newer antibiotics may have eradicated the organisms

resistant to co-trimoxazole leading to the susceptibility experienced in this study.

That these non-aureus species are resistant to a number of antibiotics is a concern. Should they infect humans, treatment may be a herculean task especially in the case of *S. lugdenensis.* While it may seem that only one of *S. lugdunensis* was isolated in this study, should it have caused infection(s) in the human it was isolated from, the drugs to treat are indeed very limited and the two (erythromycin and vancomycin) to which it was susceptible if not accessible, the human may be at risk of death. Another concern is that should the resistant gene be carried on a transmissible genetic element; such resistance could be easily spread to other organisms.

Erythromycin and vancomycin showed high levels of activity against all test organisms though not all isolates were susceptible. Other antibiotics are effective to varying extent ranging from about 12.5 – 56.3 % of the isolates. For the organisms to have developed resistance to a host of antibiotics, it shows some level of abuse, and misuse. These isolates were obtained from healthy volunteers which implies they are carriers and even though they may not be infected by them, the volunteers can transfer to other humans who may now be infected. At such a time, the organism's level of resistance may have increased. And in cases of infections by such multidrug resistant isolates, options for treatment may be limited. In a study by Heldt and Cohen[22], *S. lugdunensis* was susceptible to more antibiotics. However, in this study, *S. lugdunensis* was susceptible to only 2 out of 11 antibiotics used. This study buttresses the need to ensure antibiotic stewardship even in agricultural and veterinary settings.

The transmission of staphylococcus from animals to humans has been explained by the concept of common host-switching events between humans and animals and amongst animals due to domestication and/or commercialisation of pets and other animals. Host-switching is believed to be followed by subsequent adaptation through acquisition and/or loss of mobile genetic elements such as phages, pathogenicity islands and plasmids as well as further host-specific mutations which then allows it to spread into new host populations[23]. Due to the reported widespread sources of Staphylococci as a source of infection, it has become vital to identify them both at genus and specie level. Identifying the species is pivotal in human and veterinary medicine, for assessing the source of infection, characterizing the pathology, and conducting epidemiological studies. Prompt and accurate identification and speciation of Staphylococci is vital, not only for medical, veterinary, and societal applications but also for public health[24].

In theory, Staphylococci are gram-positive aerobic or facultative anaerobic bacteria that are nutritionally undemanding and catalase positive whereas some species producing coagulase (such as *S. aureus, S. hyicus, S. intermedius* and *S. schleiferi sub sp. coagulans*) are potentially pathogenic. Consequently, isolation of these pathogenic organisms in healthy volunteers is a health concern and proper identification and antibiotic stewardship is paramount.

CONCLUSION

This study showed the presence of animal-associated *Staphylococcus* species in human urine. Their isolation from the urine of healthy human volunteers in an academic environment is of public health concern as there is a need to minimize transmission, prevent development of infections in carriers and to prevent further transmission, especially of resistant clones that tend to spread effortlessly. The study also showed that these animal-associated organisms may be mistaken for SA or MRSA in routine checks in clinical settings. Efforts should be made to ensure the aureus and non-aureus staphylococci are correctly identified. They should no longer be labelled as contaminant and non-virulent. In addition, the isolates were resistant to a good number of the commonly prescribed antibiotics. This emphasizes the need for antibiotic stewardship in all settings – human, veterinary and agricultural.

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The authors declare that there is no conflict of interest. This study was self-funded.

REFERENCES

1. Salyer SJ, Silver R, Simone K and Barton Behravesh C. Prioritizing Zoonoses for Global Health Capacity Building-Themes from One Health Zoonotic Disease Workshops in 7 Countries, 2014-2016. Emerg Infect Dis., 2017;23(13):S55-S64.
2. WHO. Zoonotic disease: emerging public health threats in the Region. [online] 2014; https://www.emro.who.int/pdf/about-who/rc61/zoonotic-diseases.pdf?ua=1 [Accessed; 16th May 2020)
3. Bisdorff B, Scholhölter J, Claussen K, Pulz M, Nowak D and Radon K. MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany. Epidemiology & Infection, 2012;140(10):1800-1808.
4. Skov R, Christiansen K, Dancer SJ, Daum RS, Dryden M, Huang Y *et al.* Update on the prevention and control of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Int J Antimicrob Agents, 2012;39(3):193-200.
5. Loeffler A and Lloyd D. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiology & Infection, 2010;138(5):595-605.
6. Huijsdens XW, Van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN *et al.* Community-acquired MRSA and pig-farming. Annals of clinical microbiology and antimicrobials, 2006;5:1-4.
7. Wulf M, Sørum M, Van Nes A, Skov R, Melchers W, Klaassen C *et al.* Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study. Clinical Microbiology and Infection, 2008;14(1):29-34.
8. Olorunfemi PO, Onaolapo JA and Ibrahim YK. Prevalence and antibiotic susceptibility of community acquired methicillin resistant *Staphylococcus aureus* from healthy students of University of Jos. Journal of Pharmacy & Bioresources, 2020;17(2):131-141.
9. Youn J, Hwang S, Kim S, Koo H, Shin S, Moon B *et al.* mecA gene transferrability and antibiogram of zoonotic *Staphylococcus intermedius* from animals, staff, and the environment in animal hospitals in Korea. Journal of microbiology and biotechnology, 2010;20(2):425-432.
10. Sirmatel F, Yetkin G, Sirmatel P and Guzel N. Infective endocarditis in native value by *Staphylococcus xylosus*: case report and review. Case Study Case Rep., 2015;5:142-148.
11. Jindal A, Shivpuri D and Sood S. *Staphylococcus schleiferi* meningitis in a child. Pediatr Infect Dis., J 2015;34(3):329.
12. Jiang S, Zheng B, Ding W, Lv L, Ji J, Zhang H *et al.* Whole-genome sequence of Staphylococcus hominis, an opportunistic pathogen. J Bacteriol., 2012;194(17):4761-4762.
13. Chao S, Lee C, Nien C, Tzeng S and Lin H. Feline-transmitted *Staphylococcal hyicus* endophthalmitis after clear lens extraction: a case report. International Medical Case Reports Journal, 2017;10:279-283.
14. Garg S. *Staphylococcus cohnii*: not so innocuous. Journal of Acute Disease, 2017;6(5):239-240.
15. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

16. Crossley KB, Jefferson KK, Archer GL and Fowler Jr VG. eds. Staphylococci in human disease. 2nd ed. US. John Wiley & Sons, 2009.
17. Chambers HF. Community-associated MRSA-resistance and virulence converge. N Engl J Med., 2005;352(14):1485-1487.
18. Garza-González E, Morfin-Otero R, Martínez-Vázquez MA, Gonzalez-Diaz E, González-Santiago O and Rodríguez-Noriega E. Microbiological and molecular characterization of human clinical isolates of *Staphylococcus cohnii, Staphylococcus hominis*, and *Staphylococcus sciuri*. Scand J Infect Dis., 2011;43(11-12):930-936.
19. Correa J, De Paulis A, Predari S, Sordelli D and Jeric P. First report of qacG, qacH and qacJ genes in *Staphylococcus haemolyticus* human clinical isolates. J Antimicrob Chemother., 2008;62(5):956-960.
20. Piette A and Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol., 2009;134(1-2):45-54.
21. Casanova C, Iselin L, von Steiger N, Droz S and Sendi P. *Staphylococcus hyicus* bacteremia in a farmer. J Clin Microbiol., 2011;49(12):4377-4378.
22. Heldt Manica LA and Cohen PR. *Staphylococcus lugdunensis* infections of the skin and soft tissue: a case series and review. Dermatology and therapy, 2017;7:555-562.
23. Haag AF, Fitzgerald JR and Penadés JR. *Staphylococcus aureus* in Animals. Microbiology spectrum, 2019;7(3):7.3.11.
24. Irlinger F. Safety assessment of dairy microorganisms: coagulase-negative staphylococci. Int J Food Microbiol., 2008;126(3):302-310.