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ORIGINAL ARTICLE

Prevalence and Antimicrobial Resistance Pattern of Coagulase Negative Staphylococci Isolated from Pigs and in-Contact Humans in Jos Metropolis, Nigeria

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SUMMARY

The nasal carriage and antibiotic susceptibility pattern of coagulase negative staphylococci (CoNS) isolates from healthy pigs and in-contact humans were investigated in 300 pigs and 101 in-contact humans from pig farms and abattoir. The results indicate that 13.2% (53/401) of the isolates were CoNS species based on confirmatory test with Microgen biochemical kit and were further subjected to antibiotic susceptibility testing. Nine CoNS species were identified; *S. haemolyticus* (n = 10), *S. simulans* (n =5), *S. chromogen* (n = 9), *S. warneri* (n =5), *S. xylosus* (12), *S. epidermidis* (n = 5), *S. schleiferi* (n = 5) and *S. hominis* (n = 2). Overall, resistance to beta-lactams was the most observed; 79.2% of the isolates were resistant to penicillin while 11.3% and 7.5% were respectively resistant to oxacillin and cefoxitin, respectively. Resistance to 2 or more antimicrobial agents was observed in 39.6% (21/53) of the CoNS isolates. All the isolates were resistant to at least one antimicrobial agent except for gentamicin. Multidrug resistant CoNS are common colonizers of apparently healthy pigs and in-contact humans in the study area with *S. xylosus* and *S. haemolyticus* being the major species detected.

Key words: Antibiotics, CoNS, pigs, humans.

INTRODUCTION

Antimicrobial resistance is an important public health concern worldwide (Yurdakul *et al*., 2013) in both animals and humans. Development of antibiotic resistance both in human and animal bacterial pathogens has been associated with the extensive and indiscriminate use of antibiotics as growth enhancer in food animal production. The genus *Staphylococcus* is basically divided into coagulase positive or coagulase negative species based on their ability to coagulate plasma (Pyorala and Taponen 2013). The coagulase negative species constitute the majority of the genus and are opportunistic pathogens that can be isolated from the skin or mucous membrane flora of humans and animals (He *et al*., 2013). They can serve as reservoir for resistance genes

and may enhance the distribution of resistance genes into different staphylococcal species or even other bacteria genera (Shen *et al*., 2013).

Over the past years, due to the extensive use of antimicrobials in public health and animal husbandry, the antibiotic resistance of coagulase-positive staphylococci, especially some CoNS species has dramatically increased (Podkowik *et al*., 2013). Antibiotics are the first line of defense in treating clinical infections in both man and animals, and thus bacterial resistance to antibiotics is of public health concern. The use of antibiotics and other antimicrobial agents throughout the food chain contributes to the emergence of resistant bacteria that can be passed directly to humans after ingestion.

The ubiquitous nature of the genus *Staphylococcus* generally and specifically coagulase negative staphylococci enhances opportunities for infection (Davis *et al*., 2013) and furthermore the transmission of antibiotic resistance genes. CoNS species are reported to have the ability to develop resistance against a wide spectrum of antibiotics. Some CoNS that colonize animal skin and mucous membrane as flora are now being implicated in skin and soft tissue infection of man, bacteremia, and septicemia (D'mello *et al*., 2008).

Methicillin-resistant staphylococci, particularly *Staphylococcus aureus*, have been reported in pigs in many European, American and Asian countries. Also, human colonization and infections with methicillin-resistant staphylococci (MRS) have been reported in several parts of Nigeria (Fusi-Ngwa *et al*. 2007; Olowe *et al*., 2007; Ghebremedhin *et al*., 2009). However, no information exists on the occurrence of CoNS from pigs and in-contact humans in Nigeria. This study was conducted to determine the occurrence and resistance phenotypes of CoNS in pigs and in-contact humans in Jos metropolis, Nigeria.

MATERIALS AND METHODS

Sampling and Bacterial Isolation

The present study was carried out in Jos Metropolis with samples collected from pig farms and the abattoir. Samples (n=401) were randomly obtained from pigs (300) and in-contact humans (pig farmers, abattoir workers and pork sellers n=101). Ethical clearance was obtained from the Ministry of Health, Jos, Plateau State, Nigeria. A single nasal swab was collected from each of the study subject. The swabs were inoculated into 5ml Brain-heart infusion broth containing 6.5% NaCl for enrichment. After 24 h of incubation at 37°C, a loopful of broth was inoculated onto Baird Parker agar supplemented with egg yolk tellurite and incubated for between 24 h-48 h at 37ºC, following which suspected *Staphylococcus* isolates were Gram-stained and tested for coagulase, sugar fermentation and hemolysis. Subsequently the isolates were confirmed using a biochemical kit (Microgen IDSTAPH, UK). In brief, An homogenous suspension was prepared by mixing 2-5 isolated colonies in 3ml staphylococci suspending medium provided in the kit (Microgen Bioproducts, U.K), then four drops of the bacterial suspension were added to each well with a Pasteur pipette; well no 7 indicated by a black circle on the test strip coated with arginine was overlaid with two drops of mineral oil. The inoculated test strips were incubated at 35°C ± 2ºC for 24hr. After incubation, two drops of fast blue reagent was added to well no 12 indicated by a green circle on the test strip (Beta galactosidase). A colour change within 5-10seconds to plum purple was observed for positive test. All results were recorded on the organism ID report forms provided in the kit and interpreted using the Microgen identification package.

Antibiotic Susceptibility Testing

According to the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2011) the disc diffusion method was performed with the use of commercially prepared antimicrobial sensitivity disc from Oxoid UK. Isolates were tested for their antibiotic susceptibility patterns using the disk diffusion method (DDT) for 12 antibiotics by single disk obtained method (Bauer *et al*., 1966). The following antibiotics were used: ampicillin (10µg), cefoxitin (30µg), gentamicin (10µg), oxacillin (1µg), penicillin G (10units), sulphamethoxazole/trimethoprim (25µg), tetracycline (30µg) and chloramphenicol (30µg). In literal terms, multi-drug-resistance (MDR) means 'resistant to more than one antimicrobial' (Magiorakos *et al.*, 2012).

RESULTS

Out of the 401 samples collected, 53 *Staphylococcus* species were identified as coagulase negative; 27 from humans and 26 from pigs. The following eight (8) different CoNS species were identified; *S. haemolyticus* 18.9% (10/53), *S. xylosus* 22.6% (12/53) and *S. chromogen* 16.9% (9/53). *S. simulans, S. warneri, S. schleiferi* and *S. epidermis* all had 9.4% (5/53) respectively while *S. hominis* showed the least percentage occurrence [3.8% (2/53)] as shown in Table 1. The result showed that most of the species were resistant to penicillin, tetracycline and trimethoprim while a few were resistant to chloramphenicol, oxacillin, cefoxitin and gentamicin. Among the isolates, 39.6% were resistant to 3 or more antimicrobial agents tested. The in vitro antimicrobial resistance profile of all the CoNS isolates tested are summarized in Table II. Table III summarizes the number of isolates resistant to 2 or more antimicrobial agents tested. Of the 53 isolates tested, 39.6% (21/53) were resistant to 2 or more of the 10 antimicrobial agents tested. All the isolates showed almost similar resistance patterns. Although *S. haemolyticus* showed a considerable rate of resistance to 3 different patterns, generally, 2 type of patterns were most occurring: PEN; PEN, TET and PEN, TET, SXT.

DISCUSSION

This study showed that the prevalence of CoNS carriage among pigs and in-contact humans were 8.6% and 26.7% respectively while the overall prevalence was 13.2%. The eight different species identified showed marked multidrug resistance to the antimicrobial agents tested against them. All the isolates showed a high resistance to the beta-lactams and this result is in agreement with the report of Detwiller *et al*., (2013) who reported that CoNS are often resistant to beta-lactams. Despite the low level of methicillin resistant coagulase negative staphylococci (MRCONS) 7.5% observed in the study, it is still of public health importance. MRCONS have been considered to be a source for horizontal gene transfer of resistance gene to *S. aureus* (Zhang *et al*., 2009) and interspecies transfer have also been documented (Swenson *et al*., 2009). Apart from the beta-lactams, most of the isolates were also resistant to 2 or more antimicrobial agents. This could be associated with the fact that CoNS are often reported as methicillin resistant with co-resistance to different classes of antimicrobials (Huber *et al.*, 2011).

The problems associated with CoNS can be worsened by the notorious escalation of antibiotic resistance observed during the last decades in these bacteria (Yurdakul *et al*., 2013). Even though CoNS opportunistic infections are most of the times regarded as being of environmental origin, the distribution and antibiotic resistance patterns of these organisms in the environment are poorly understood. This resistance pattern is quite worrisome because some CoNS may

TABLE 1: Distribution of Coagulase Negative *Staphylococcus* (CoNS) among humans and pigs

CoNS	Humans (%)	Pigs (%)	
S. haemolyticus	8 (80)	2 (20)	
S. simulans	2 (40)	3 (60)	
S. warneri	3 (60)	2 (40)	
S. schleiferi	2 (40)	3 (60)	
S. xylosus	4 (33.3)	8 (66.7)	
S. chromogen	1 (11.1)	8 (88.9)	
S. epidermidis	5 (100)	0 (0)	
S. hominis	2 (100)	0 (0)	
Total	27	26	p<0.0001

TABLE II: Prevalence of antimicrobial resistance by species of Coagulase Negative staphylococci isolates from humans and pigs

Antibiotics	No of Resistant staphylococci species (%)							
	S. haemolyticus(n = 10)	*S. simulas* (n = 5)	*S. warneri* (n = 5)	*S. schleiferi*(n = 5)	*S. xylosus* (n =12)	*S. chromogens* (n = 9)	*S. hominis* (n = 2)	*S. epidermidis* (n = 5)
Penicillin	8 (80)	3 (60)	4 (80)	2 (40)	6 (50)	9 (100)	2 (100)	4 (80)
Tetracycline	5 (50)	2 (40)	1 (20)	2 (40)	3 (25)	5 (56)	2 (100)	0 (0)
Trimethoprim/sulphamethoxazole	4 (40)	0 (0)	1 (20)	1 (20)	2 (16.5)	4 (44)	1 (50)	0 (0)
Chloramphenicol	1 (10)	0 (0)	0 (0)	1 (20)	2 (16.5)	1 (11)	0 (0)	0 (0)
Gentamicin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oxacillin	0 (0)	0 (0)	0 (0)	1 (20)	3 (25)	2 (22)	0 (0)	0 (0)
Cefoxitin	0 (0)	0 (0)	0 (0)	0 (0)	3 (25)	1 (11)	0 (0)	0 (0)

TABLE III: No of CoNS isolates resistant to 2 or more antimicrobials and the resistance patterns most frequently observed among the isolates

CoNS species (n = 8)	No of isolates tested	No of isolates resistant to 2 or more drugs (%)	No of Pigs	No of Humans	Pattern types	No of isolates (%)
S. haemolyticus	10	5 (50)	3	2	PEN, TET PEN, TET, SXT	4 (80) 1 (20)
S. simulans	5	1 (20)	1	0	PEN, TET	1 (100)
S. warneri	5	1 (20)	0	1	PEN, TET	1 (100)
S. schleiferi	5	2 (40)	1	1	PEN, TET	2 (100)
S. xylosus	12	5 (42)	3	2	PEN, TET PEN, TET, SXT	3 (60) 2 (40)
S. chromogen	9	6 (60)	2	4	PEN, TET, SXT,	6 (100)
S. hominis	2	1 (50)	0	1	PEN, TET	1 (100)
S. epidermidis	5	0 (0)	0	0	NONE	
Total	53	21 (39.6%)				

act as opportunistic pathogens by colonizing exposed wounds of carriers and can as well transfer antibiotic resistance genes to other microorganisms either horizontally or vertically. *S. haemolyticus* and *S. epidermidis* were among the CoNS species identified in this study and they have been reported to cause infection in human (Anthony and Okoh 2014). Also, previous study has reported *S. haemolyticus* in pigs (Anthony and Okoh 2014). Tulinski *et al*. (2012) reported *S. haemolyticus* to be the highest CoNS species to be isolated from a pig farm. *S. epidermidis* has been reported as one of the most important causes for hospital-acquired bacteremia and found many isolates from hospitalized patients to be methicillin-resistant.

Recently, several authors reported MRCONS also in healthy animal (Huber *et al*., 2011) but in this study it was mostly isolated from humans and this further explains it full establishment as mainly a human colonizer. *S. schleiferi* was also one of the species isolated in this study and it is of public health concern because it has been reported to be an emerging potential zoonotic pathogen (Davis *et al.,* 2013). Typically they have been associated with skin infections in companion animals (otitis or pyodermal in dogs) and cats and has recently been described as a human pathogen (Kumar *et al*., 2007; Tzamakis *et al*., 2013).

In this study, 3 out of the 5 *S. schleiferi* isolates were from pigs while the other 2 were from humans. Also, asymptomatic nasal or skin carriage in people with veterinary occupational contact has been demonstrated with prevalence ranging between ≤ 2% to 50% (Vanni *et al*., 2009; Ishihara *et al*., 2010; Morris *et al*., 2013; Cain *et al*., 2011a; Cain *et al*., 2011b; Penna *et al*., 2013). This species also showed

multidrug resistance and this is in agreement with the reports of the following workers (Starlander *et al*., 2011; El-Jakee *et al*., 2013) who reported multidrug resistance of this species.

Generally, most of the isolates showed high resistance to penicillin, tetracycline, trimethoprim, and chloramphenicol. The reason for this resistance could be associated with the fact that most of the antibiotics are commonly used for treatment of infection and as growth performance enhancer and since more than one species of microorganism can be present within a host there is therefore the possibility of acquiring resistance. They could also have acquired the resistance from the environment since they are backyard raised pigs. The importance of most of these species is not fully understood especially in pigs and this may be as a result of the fact that most reports have focused on surgical site infections in humans and little is known in animals especially pig. However, most of these CoNS species have been frequently associated with mastitis in animals (El-Jakee *et al*., 2013) and bacteremia in humans. Even though some of these CoNS species have limited zoonotic potential they should be carefully monitored so as not to reduce therapeutic options for treatment of their infections in both humans and animals as a result of multidrug resistance.

CONCLUSION

This study therefore, highlight the presence of CoNS especially the multidrug resistant strains. It also showed marked antibiotic resistance pattern of the isolates to different antimicrobial agents that are frequently used both in human and veterinary medicine. It is therefore suggested that monitoring of antibiotic profile among CoNS in livestock and in-contact humans is necessary for the control of spread of these strains to the community and hospital settings.

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