

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN SELECTED PIG FARMS IN NEKEDE, OWERRI/IMOSTATE.

'Umeh, S.I. and 'Otorogu U.F.

Department of Microbiology, Federal University of Technology,
P.B.M.1526 Owerri, IMO -State, Nigeria.

Abstract: In recent years, a negative trend of increasing prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) strains has been noted worldwide. Cases of MRSA have increased in livestock animals and are found in intensively reared production animals where it can be transmitted to humans. The study investigated the occurrence of MRSA in pigs from selected piggery farms. In Nekede with high density of pig production. Out of the 200 nasal and rectal swabs collected MRSA was isolated from 68% of the pigs. Isolated were confirmed to be *Staphylococcus aureus* on the basis of colonial morphology, coagulase production, DNase and catalase tests. Antimicrobial pattern of *S. aureus* showed resistance to levofloxacin (59%) ciprofloxacin (54%), methicillin (91%), ampiclox (85%) and amoxicillin (69%). This study demonstrated the prevalence of MRSA in pigs from these farms and it is a concern because of the transmission of MRSA between pigs and humans.

Key words: *Staphylococcus aureus*, MRSA swine, Resistance, Zoonosis.

INTRODUCTION

Staphylococcus aureus is a facultative anaerobic, gram- positive coccus that belongs to the family *micrococcaceae* and it is catalase positive and oxidase negative.

Methicillin- resistant *Staphylococcus aureus* (MRSA) includes those strains that have acquired a gene giving them resistance to methicillin and essentially all other beta lactam antibiotics

Pigs, cattle and poultry are colonized with MRSA and the zoonotic transmission of such MRSA to humans via direct animal contact, environmental contaminations of meat are a matter of concern (Kock *et al*, 2014). Besides direct exposition to livestock, potential sources for transmission include farm visits or farm workers (Cuny *et al*, 2009 and Bisdorff *et al*, 2011).

Airborne emission of MRSA from livestock farms have been reported but only in very low concentrations (Schulz *et al.*, 2012).

Methicillin –resistant *Staphylococcus aureus* can be transmitted between people and animals during close contact and frequently infections in companion animals can be traced back to their human caretakers. This study investigated the occurrence of methicillin- resistant *Staphylococcus aureus* among pigs from selected piggery farms in Nekede Owerri, Imo- State.

MATERIALS AND METHOD

Sample collection: This study was conducted based on randomly selected piggeries between the period of April- July, 2013 in Neke'e, Owerri in Imo-State. A total of 200 rectal and nasal swabs were collected from pigs of different age groups (piglets, grower finisher hogs). All samples were collected using cotton tipped swabs that were placed in liquid Stuart's medium.

*Corresponding author:

'Umeh, S.I

Copyright © 2015 Nigerian Society for Microbiology

Isolation and identification of bacteria:

Swabs were inoculated into enrichment 1 broth and incubated at 37°C for 24h. A loopful of vortexed broth was inoculated onto selective MRSA agar plates (MRSA select, Bio Rad, Hercules, CA). The plates were then incubated aerobically at 37°C for 24-48h. The colonies were tested and confirmed to be *Staphylococcus aureus* by subculturing on mannitol Salt Agar, which is a selective medium for *Staphylococcus aureus* and then by using conventional methods that include gram-staining, colonial morphology, coagulase, DNase and catalase tests.

Antibiotic susceptibility testing: Isolates were tested for susceptibility to different

antimicrobials by using the disk diffusion method of Kirby-Bauer recommended by World Health Organization. The antimicrobials used were ciprofloxacin (5µg), norfloxacin (10µg), gentamycin (10µg), amoxicillin (20µg), streptomycin (10µg), rifampicin (30µg), erythromycin (15µg), chloramphenicol (30µg), ampiclox (10µg) levofloxacin(5µg) and methicillin (5µg). The diameter of the zone of inhibition for each antimicrobial was measured in mm using a meter ruler. The inhibition zone was interpreted using the interpretative chart as described by the Clinical Laboratory Standards Institute (CLSI) guidelines and reported as resistance, intermediate or susceptible.

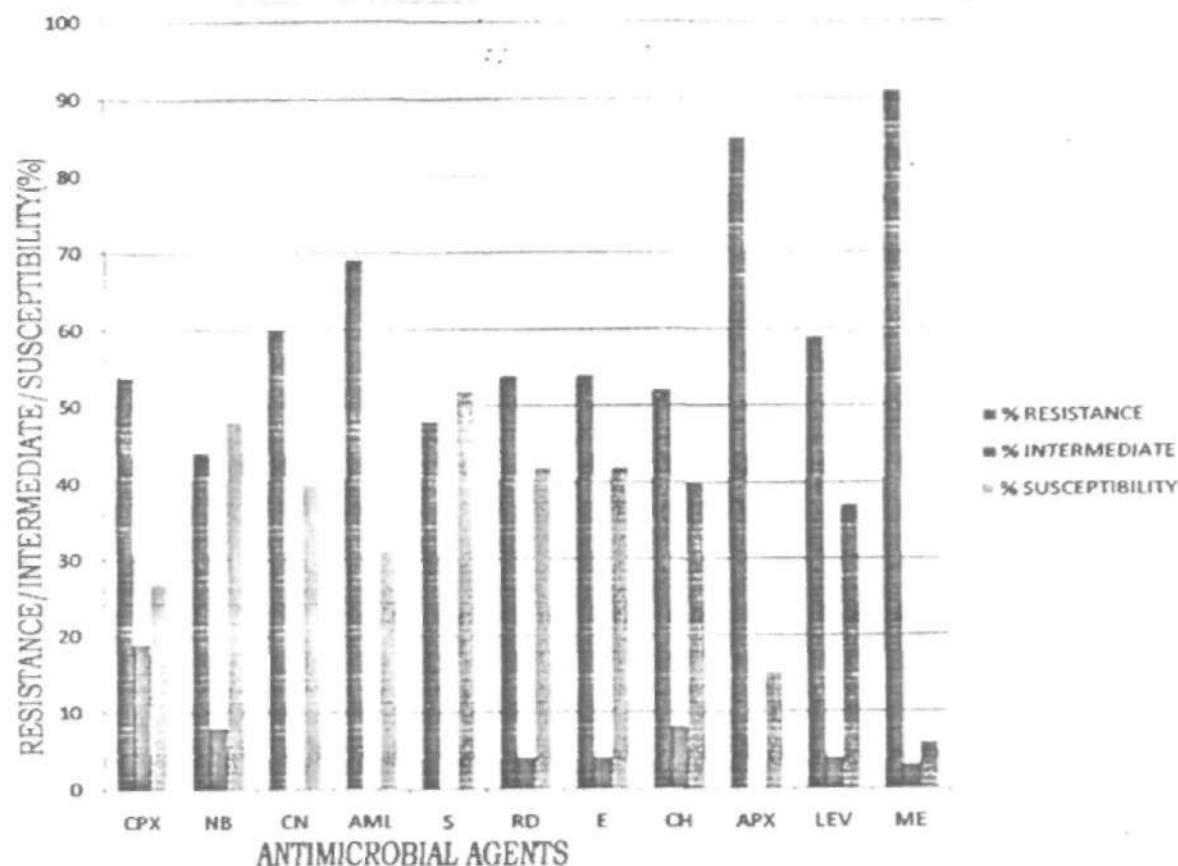
RESULTS

Out of the 200 nasal and rectal swabs from pigs, MRSA was isolated from 68% of the pigs. The result of the antimicrobial susceptibility testing is shown in table 1.

Table1: Antimicrobial Susceptibility testing of *Staphylococcus aureus* from pigs

Antimicrobial Potency	Disc Potency(µg)	Resistance%	Intermediate%	Susceptibility %
Ciprofloxacin	5	54	19	27
Norfloxacin	10	44	8	48
Gentamycin	10	60	0	40
Amoxicillin	20	69	0	31
Streptomycin	10	48	0	52
Rifampicin	30	54	4	42
Erythromycin	15	54	4	42
Chloramphenicol	30	52	8	40
Ampiclox	10	85	0	15
Levofloxacin	5	59	4	37
Methicillin	5	91	3	6

As shown in table 1, the isolates were susceptible to streptomycin, norfloxacin, erythromycin and rifampicin, but highly resistant to methicillin ampiclox, amoxicillin, gentamycin levofloxacin with least activity against norfloxacin.



DISCUSSION

This study detected MRSA in pigs from selected farms in Nekede, Owerri, Imo state. The presence of MRSA in pigs are of great concern because pigs are food producing animals and there are possibilities of food contamination and also their presence may pose a public health risk to human contacts.

A total prevalence of 68% was found in this study. Pletinckx *et al.* (2011) reported a higher prevalence varying between 82% and 92%. Also a higher isolation rate of 83% was reported by (Isabelle *et al.*, (2011); Khanna *et al.*, (2008) and Huijsdens *et al.*, (2006). The high prevalence of MRSA reported in this study demonstrates that MRSA is common among pigs in the study area. This may be due to the fact that the pigs were grouped together and

lived in close contact, facilitating the spread of MRSA.

In this study, all MRSA isolates were resistant to Methicillin (91%), Ampiclox (85%), Amoxicillin (69%), Gentamicin (60%), Levofloxacin (59%) and Ciprofloxacin (54%) with least resistance to Norfloxacin (44%) Streptomycin (48%). This is in agreement with previous studies by (Vos *et al.*; (2005); Willems *et al.*; (2007); and De Neeling *et al.*; (2007) who reported multidrug resistance pattern in MRSA among pigs.

The high resistance obtained from these drugs may be due to indiscriminate use of drugs in pig production. Some of the antimicrobials are broad-spectrum drugs frequently used for growth promotion and treatment of bacterial infections thus, the isolates may have been exposed to

the antibiotics resulting in development of resistance against them.

The resistance patterns exhibited by the isolates proved further that the isolates were resistant to three or more of the drugs tested. It is well documented that *MecA* gene which encodes for methicillin resistance in *Staphylococcus*, also encodes for resistance to other classes of antimicrobial agents.

CONCLUSION

This study demonstrated MRSA colonization in pigs in the study area. Monitoring the emergence of resistant pathogens in pig population is important particularly because of their zoonotic potentials. Further surveillance of the epidemiology of MRSA in pigs and humans, preventive measures at the farm level and antibiotic stewardship are advised.

REFERENCES

- Bisdorff, B., scholholter, J.L., Claussen, k., pulz, M. and Nowalk, D. (2011). MRSA ST398 in livestock farmers and neighboring residents in a rural area in Germany. *Epidemiol infect* 140:1800-1808.
- Cuny, C., Nathaus R., Layer,F., Strommenger, B., and Altmann, D .(2009).Nasal colonization of humans with methicillin – resistant *Staphylococcus aureus* (MRSA)CC 398 with and without exposure to pigs. *PLos one* 4: e6800. Do I : 10. 1371/ Journal pone 0006800.
- De Neeling , A. J ., Van den Broek , M. J. M. and Sparlburg E. C. (2007). High prevalence of methicillin resistant *Staphylococcus aureus* in pigs . *Vet Microb*. 122:336-72.
- Huijsdens, X. W, Van Dijke, B. J. and spalburg, B.J. (2006). Community – acquired MRSA and pig farming .*Ann Clun Microbial Antimicrob*. 5:26 .
- Isabelle, D., Winy, M., Tngrid , D. Pierre, D. , Lieve , H., Patrick B., Marc, H. and Geertrui, R. (2011).Characterization of methicillin resistant *Staphylococcus aureus* on two Belgian pig farms. *Vet Sci. Dev* 1(1) doi :10. 4081/vsol.2011.e1
- Khanna, T., Friendship R., Dewey, C. and weese J.S.(2008). Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Vet Microbiol* .298-03.
- Kock , R., Loth, B., koksa M. , Schulte Wulwer, J and Harlizus, J. (2012). Persistence of nasal colonization with livestock- associated methicillin resistant *Staphylococcus aureus* in pig farmers after holidays from pig exposure. *Appl Environ Microbio*. 78: 4046- 4047.
- Pletinckx, L.J, Verhegghe, M., Dewulf, J., Crombe, F. , Bleecker, Y.D. , Rasschaert, G., Godderis , R. M. and Man , D.T. (2011). Screening of poultry – pig farms for methicithin – Resistant *Staphylococcus aureus*: Sampling methodology and within Herd prevalence in Broiler Flocks and pigs. *Infection , Genetics and Evolution* 11(8): 2133-7 .
- Schulz,J., Friese, A, Klees, S., Tenhagen, B.A. and Fetsh,A.(2012). LA-MRSA contamination of air and soil surfaces in the vicinity of pig barns: A longitudinal study. *Appl Environ Microbiol* 78:5666-5674
- Vos A , Loeffeen, F. and Bakker, J. (2005). Methicillin resistant *Staphylococcus aureus* in pig farming .*Emerg Infect Dis*. 11: 1965-6.
- Willems , G , Dispas , M and Denis O. (2007). Characterization of MRSA from pigs in Belgium. 2nd Symposium on Antimicrobial Resistance in Animals and the Environment (AREA) , Tours ,France.