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Research Article
Antibiotic Susceptibility of Fastidious and Non-fastidious Bacteria from African Swine Fever Pigs to Standard Antibiotics and 'Luwine'

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Abstract

Background and Objective: African swine fever is a highly contagious viral disease that affects domestic pigs and it is characterized by fever, blotching of skin, hemorrhage of the lymph nodes, internal organs and the gastrointestinal tract. Management of viral diseases require the immune system of the organism and the use of antibiotics to control secondary infections. This study determined the antibiotic susceptibility of fastidious and non-fastidious bacteria isolated from pigs infected with African swine fever to standard antibiotics and 'Luwine' (a local herbal medicine prepared from the root of *Sarcocephalus latifolius* and dry bark peels of *Pseudocedrela kotschyi* used by farmers to control Africa swine fever in Navrongo, Ghana. **Materials and Methods:** The disc diffusion method was used for the antibiotic susceptibility test. The non-fastidious and fastidious bacteria were tested against 8 antibiotics and 'Luwine'. **Results:** The non-fastidious and fastidious bacteria were all resistant to 'Luwine'. The non-fastidious bacteria were highly susceptible to azithromycin (100%), gentamicin (100%) and amoxycillin/clavulanic acid (80%) but resistant to teicoplanin (70%). The fastidious bacteria were highly susceptible to gentamicin (70%) and sulfamethoxazole/trimethoprim (70%) but resistant to ceftriaxone (100%), teicoplanin (100%) and chloramphenicol (80%). Intermediate resistant occurred for all the antibiotics except azithromycin and gentamicin for non-fastidious bacteria. Intermediate resistant also occurred for all the antibiotics except ceftriaxone and teicoplanin for fastidious bacteria. Multidrug resistant occurred between the non-fastidious (50%) and fastidious (90%) bacteria. The non-fastidious bacteria exhibited 8 different antibiotic resistant patterns. The fastidious bacteria exhibited 10 different antibiotic resistant patterns. Multiple antibiotic index (MAR) ranged from 0.13-0.50 and 0.25-0.75 for non-fastidious and fastidious bacteria, respectively. **Conclusion:** The non-fastidious bacteria were generally more resistant than the fastidious bacteria. 'Luwine' was not effective against the bacteria associated with African swine fever infection. Among the antibiotics, gentamicin was the best for controlling bacteria associated with African swine fever pigs.

Key words: Antimicrobial resistance, herbal medicine, microorganisms, swine diseases, non-fastidious, African swine fever

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

African swine fever is a fatal viral disease of pigs caused by a large DNA virus of the Asfarviridae family and replicates in the cytoplasm[1]. The African swine fever virus is highly resistant in the environment, especially at low temperatures and to many disinfectants[2]. It is easily spread between pigs by direct contact with an infected animal, its body fluids (nasal, oral, feces, blood) or tissues (meat) or indirectly from contact with contaminated objects (fomites) such as vehicles, equipment, footwear or clothing[2,3]. The management of African swine fever could include the use of antibiotics to treat secondary infections.

Antibiotics have been widely used in animal production since antiquity. They have been used in livestock production for purposes such as therapeutic use to treat sick animals; metaphylaxis or short-term medication to treat diseased animals and to prevent infection in other animals; prophylactic use to prevent infections at times of risk such as transport or weaning and growth promotion to improve feed utilization and production[4-8]. Their use in animal production has been attributed in part to increase bacteria resistance[5,6]. Various types of non-fastidious and fastidious bacteria play a role in complicating diseases in pigs. King[9] reported that disease causing organisms and diagnosis of infections are very important health constraints, especially when they involve fastidious organisms which are very difficult to culture in the laboratory.

African swine fever is quite rampant and has been responsible for the death of many pigs in the Upper East Region of Ghana. In order to curb the situation, pig farmers in this Region use 'Luwine' (a herbal medicine made from the root of African peach, *Sarcocephalus latifolius* and dry bark peels of African dry zone ceder, *Pseudocedrela kotschyi*) as treatment for African swine fever. Secondary infections in pigs as a result of African swine fever is also possible. Therefore, this study was carried out to ascertain the effectiveness of 'Luwine' and standard antibiotics against bacteria associated with secondary infections in African swine fever pigs.

MATERIALS AND METHODS

Experimental plants, sites and duration: The plants (African peach, *Sarcocephalus latifolius* and African dry zone ceder, *Pseudocedrela kotschyi*) used for 'Luwine' preparation were obtained from Bongo Soe, Upper East Region, Ghana. 'Luwine' preparation was also done in Bongo Soe by a pig farmer. Antibiotic susceptibility test was carried out at the Spanish Laboratory of the University for Development Studies, Nyankpala Campus, Ghana. The experiment was conducted between May and June, 2019.

Preparation of 'Luwine': The root of African peach (*Sarcocephalus latifolius*) weighing 0.38 kg and dry bark peels of African dry zone ceder (*Pseudocedrela kotschyi*) weighing 2.32 kg were boiled in 12 L of water for 1 h. After which, 0.17 kg of salt was added and boiled for further 30 min. It was then allowed to cool and decanted into sterilized bottles. About 6.5 L of 'Luwine' was obtained from the 12 L of water after boiling, cooling and decanting. It was then stored in a refrigerator at 4°C for later use.

Collection of swab samples: Sterilized swabs were used to swab the anus (n = 3), mouth (n = 3) and nose (n = 4) of pigs infected with African swine fever. The swabs were stored in an ice chest containing ice block, transported to the laboratory and analyzed immediately upon arrival at the Spanish Laboratory.

Antibiotic discs and impregnation of blank disc with 'Luwine': The standard antibiotic discs used were amoxycillin/clavulanic acid 30 µg (AMC), azithromycin (AZM) 15 µg, ceftriaxone (CRO) 30 µg, chloramphenicol (C) 30 µg, gentamicin (CN) 10 µg, teicoplanin (TEC) 30 µg, tetracycline (TE) 30 µg and sulfamethoxazole/trimethoprim (SXT) 25 µg. Blank antibiotic discs were soaked in 'Luwine' for 5 min to allow impregnation of discs with 'Luwine'.

Antibiotic susceptibility test for fastidious and non-fastidious bacteria: The antibiotic susceptibility test was done using a slightly modified method of Bauer-Kirby[10]. For the antibiotic susceptibility test for fastidious bacteria, the swabs were dipped in 10 mL sterilized Trypticase Soy Broth (TSB) and spread plated on Müller Hinton Agar (MHA). Four antibiotic discs and a blank disc impregnated with 'Luwine' were placed on the MHA at a distance to prevent overlapping of the inhibition zones. The MHA plates were incubated at 37°C for 24 h. With regards to antibiotic susceptibility test for fastidious bacteria, the swabs were dipped in sterile 10 mL TSB and incubated at 37°C for 18 h. After incubation, it was adjusted to 0.5 McFarland turbidity using sterile TSB and spread plated on MHA. The MHA plates were also incubated at 37°C for 24 h.

After incubation of the MHA plates (for both fastidious and non-fastidious bacteria), the inhibition zones were measured and the results interpreted as susceptible,

intermediate resistant or resistant according to Clinical Laboratory Standard Institute[11]. The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman[12] using the equation: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

All media and antibiotic discs used were purchased from Oxoid Limited, Basingstoke, UK.

RESULTS

Antibiotic resistance of non-fastidious bacteria: The percentage antibiotic resistance of non-fastidious bacteria is presented in Table 1. From Table 1, the non-fastidious bacteria were resistant to 'Luwine' (100%), teicoplanin (70%), ceftriaxone (40%), chloramphenicol (40%) and sulfamethoxazole/trimethoprim (40%). They were however, highly susceptible to azithromycin (100%), gentamicin (100%) and amoxycillin/clavulanic acid (80%). Moderate intermediate resistances occurred for ceftriaxone (30%), tetracycline (30%) and amoxycillin/clavulanic acid (20%).

Antibiotic resistance of fastidious bacteria: The antibiotic susceptibility of the fastidious bacteria to the antibiotics examined is shown in Table 2. All the fastidious bacteria were resistant to 'Luwine' (100%), ceftriaxone (100%) and teicoplanin (100%). Nonetheless, they were highly susceptible to gentamicin (70%) and sulfamethoxazole/trimethoprim (70%). Moderate intermediate resistances also occurred for amoxycillin/clavulanic acid (40%), azithromycin (20%) and chloramphenicol (20%).

Antibiotic resistant profile of the non-fastidious bacteria: The antibiotic resistant profile of the non-fastidious bacteria can be found in Table 3. The non-fastidious bacteria exhibited 8 different profiles, that is TecTeCroSxt, TecCCroSxt, TecCCro, TecCSxt, TecTeC, TecSxt, Tec and Cro with a multiple antibiotic index ranging from 0.13-0.50. Two non-fastidious bacteria from pig mouth sources were susceptible to all the antibiotics. Multidrug resistant (resistant to 3 or more different classes of antibiotics), was exhibited by 5 groups of non-fastidious bacteria.

Antibiotic resistant profile of the fastidious bacteria: The antibiotic resistant profile of the fastidious bacteria is shown in Table 4. The fastidious bacteria exhibited 10 different profiles, that is AmcAzmTecTeCroSxt, AzmTecCnTeCCro, AmcTecTeCCro, TecTeCCroSxt, AmcTecCCro, AzmTecCCro,

Table 1: Percentage antibiotic resistance of non-fastidious bacteria isolated from pigs affected with African swine fever

Antimicrobial	R (%)	I (%)	S (%)
Amoxycillin/clavulanic acid (AMC) 30 µg	0	20	80
Azithromycin (AZM) 15 µg	0	0	100
Ceftriaxone (CRO) 30 µg	40	30	30
Chloramphenicol (C) 30 µg	40	10	50
Gentamicin (CN) 10 µg	0	0	100
Teicoplanin(TEC) 30 µg	70	10	20
Tetracycline (TE) 30 µg	20	30	50
Sulfamethoxazole/trimethoprim (SXT) 25 µg	40	10	50
'Luwine'	100	0	0

S: Susceptible, I: Intermediate, R: Resistant, Luwine: Herbal medicine made from the root of *Sarcocephalus latifolius* and dry bark peels of *Pseudocedrela kotschyi*

Table 2: Percentage antibiotic resistance of fastidious bacteria isolated from pigs affected with African swine fever

Antimicrobial	R (%)	I (%)	S (%)
Amoxycillin/clavulanic acid (AMC) 30 µg	30	40	30
Azithromycin (AZM) 15 µg	30	20	50
Ceftriaxone (CRO) 30 µg	100	0	0
Chloramphenicol (C) 30 µg	80	20	0
Gentamicin (CN) 10 µg	20	10	70
Teicoplanin (TEC) 30 µg	100	0	0
Tetracycline (TE) 30 µg	50	10	40
Sulfamethoxazole/trimethoprim (SXT) 25 µg	20	10	70
'Luwine'	100	0	0

S: Susceptible, I: Intermediate, R: Resistant, Luwine: Herbal medicine made from the root of *Sarcocephalus latifolius* and dry bark peels of *Pseudocedrela kotschyi*

Table 3: Antibiotic resistance profile and multiple antibiotic resistance index (MAR index) of non-fastidious bacteria isolated from pigs affected with African swine fever

Code	Sources	No. of antibiotics	Antibiotic resistant profile	MAR index
A1	Anus	4	TecTeCroSxt	0.50
4A	Anus	4	TecCCroSxt	0.50
5N	Nose	3	TecCCro	0.38
4N	Nose	3	TecCSxt	0.38
2N	Nose	3	TecTeC	0.38
3N	Nose	2	TecSxt	0.25
3A	Anus	1	Tec	0.13
1M	Mouth	1	Cro	0.13
5M	Mouth	0	-	0.0
2M	Mouth	0	-	0.0

Table 4: Antibiotic resistance profile and multiple antibiotic resistance index (MAR index) of fastidious bacteria isolated from pigs affected with African swine fever

Code	Sources	No. of antibiotics	Antibiotic profile	MAR index
A1	Anus	4	TecTeCCro	0.50
3A1	Anus	4	AmcTecCCro	0.50
4A1	Anus	5	TecTeCCroSxt	0.63
3N1	Nose	6	AzmTecCnTeCCro	0.75
2N1	Nose	3	TecCCro	0.38
4N1	Nose	2	TecCro	0.25
5N1	Nose	4	AzmTecCCro	0.50
2M1	Mouth	4	TecCnCCro	0.50
1M1	Mouth	5	AmcTecTeCCro	0.63
5M1	Mouth	6	AmcAzmTecTeCroSxt	0.75

TecTeCCro, TecCnCCro, TecCCro and TecCro with a multiple antibiotic index ranging from 0.25-0.75. Multidrug resistant was also exhibited by 9 groups of fastidious bacteria.

DISCUSSION

This is the first report on the use of 'Luwine' against fastidious and non-fastidious bacteria associated with pigs infected with African swine fever. Non-fastidious bacteria are those bacteria that are able to grow and replicate without special nutrient supplements and includes *Escherichia coli*, *Listeria* species, *Pseudomonas* species and *Staphylococcus* species[13]. In contrast, fastidious bacteria are those that require special nutrient requirements and sometimes atmospheric environment to grow and replicate[9,14]. They include *Brucella* species, *Campylobacter* species, *Helicobacter* species and *Legionella* species[14]. These groups of bacteria are involved in causing illnesses, food spoilage, food poisoning and secondary infections in animals and/or humans. African swine fever is a highly contagious hemorrhagic viral infection of pigs with symptoms including high fever (41-42°C), rapid breathing, flushing skin and thick whitish discharges from the nose and eyes[15]. The disease is highly fatal (almost 100% mortality) and results in significant losses to the pig and meat industries[3,15,16].

Pig farmers in the Upper East Region of Ghana and its environs use 'Luwine' to control African swine fever. The farmers believe that 'Luwine' was effective in controlling African swine fever due to their experience with the use of this herbal preparation for treating African swine fever pigs. According to the farmers, they have observed that African swine fever pigs treated with 'Luwine' survived better than non-treated pigs. Scientifically, the cure for the virus responsible for African swine fever is yet to be discovered. The prevention and management of this disease rely on vaccination, the immune system of pigs and the use of antibiotics to control secondary infections. The rational of this work was to determine the antibiotic(s) that could help control secondary infections in pigs suffering from African swine fever. It also sought to determine whether 'Luwine' played a role in reducing secondary infections associated with African swine fever. From the results, the non-fastidious bacteria were highly susceptible to azithromycin, gentamicin and amoxycillin/clavulanic acid, while the fastidious bacteria were susceptible to gentamicin and sulfamethoxazole/trimethoprim. Thus the afore-mentioned antibiotics are recommended for the management of secondary infections associated with African swine fever. When an antibiotic is required, gentamicin will be a better option since a high proportion of both fastidious and non-fastidious organisms were susceptible to this antibiotic. A high susceptibility of *Escherichia coli*, a non-fastidious bacterium isolated from meat and drinking water for animals in Ghana to gentamicin has also been reported by Adzitey[17] and Adzitey *et al.*[18].

However, the fastidious bacteria were highly resistant to 'Luwine' and teicoplanin; while it was 'Luwine', ceftriaxone, chloramphenicol and teicoplanin for non-fastidious bacteria. These antibiotics are therefore, not recommended for the treatment of secondary infections associated with African swine pigs. Intermediate and multidrug resistances were also exhibited by the non-fastidious and fastidious bacteria. Intermediate resistant bacteria easily become resistant to antibiotics[18,19] and multidrug resistant are those bacteria that were resistant to 3 or more different classes of antibiotics[5,19]. Fifty percent of the non-fastidious bacteria exhibited multidrug resistant. As high as 90% of the fastidious bacteria exhibited multidrug resistant. Both intermediate and multidrug resistant bacteria are difficult to treat when they are involved in an infection and a major threat to the successful treatment of infectious diseases worldwide[20,21]. Non-fastidious and fastidious bacteria including *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella*, *Pneumoniae*, *Salmonella* species, *Staphylococcus aureus* resistant to multiple antibiotics have also been reported by Adzitey *et al.*[21], Elkenany[22], Ayefoumi *et al.*[23] and Abdel Azim *et al.*[24].

CONCLUSION

Antibiotics such as azithromycin, gentamicin, sulfamethoxazole/trimethoprim and amoxycillin/clavulanic acid could be used in the management of secondary infections in African swine fever pigs. When only one antibiotic is required, gentamicin will be the best choice. 'Luwine' locally used to treat African swine fever pigs was not found to be effective against the non-fastidious and fastidious bacteria associated with African swine fever pigs, therefore, it is not recommended for the treatment of secondary infections linked to African swine fever in pigs. A high proportion of the fastidious bacteria exhibited multidrug resistant as compared to the non-fastidious bacteria. *In vivo* and *in vitro* studies to investigate the anti-viral potential of the 'Luwine' is recommended.

SIGNIFICANT STATEMENTS

This study discovered that bacteria from African swine fever exhibited multidrug resistant. Nonetheless, gentamicin

can be used to control bacteria associated with secondary infections in pigs suffering from African swine fever. In the absence of gentamicin; amoxycillin/clavulanic acid, azithromycin and sulfamethoxazole/trimethoprim could be used. 'Luwine' a local herbal preparation does not control bacteria associated with African swine fever diseases.

REFERENCES

1. Sanchez-Cordon, P.J., M. Montoya, A.L. Reis and L.K. Dixon, 2018. African swine fever: A re-emerging viral disease threatening the global pig industry. Vet. J., 233: 41-48.
2. CFSPH., 2018. Fast facts: African swine fever. The Center for Food Security and Public Health (CFSPH), Iowa State University, Ames, IA., USA. http://www.cfsph.iastate.edu/FastFacts/pdfs/african_swine_fever_F.pdf
3. FAO., 2000. Recognizing African Swine Fever: A Field Manual. Food and Agriculture Organization, Rome, Italy, ISBN: 92-5-104471-6, Pages: 38.
4. Viola, C. and S.J. DeVincent, 2006. Overview of issues pertaining to the manufacture, distribution and use of antimicrobials in animals and other information relevant to animal antimicrobial use data collection in the United States. Prev. Vet. Med., 73: 111-131.
5. Mathew, A.G., R. Cissell and S. Liamthong, 2007. Antibiotic resistance in bacteria associated with food animals: A United States perspective of livestock production. Foodborne Pathog. Dis., 4: 115-133.
6. Deli, R.N.R.A. and F. Adzitey, 2017. Prevalence and antibiotic resistance of *Salmonella* serovars isolated from spent hens and its environmental samples in Penang and Kedah, Malaysia. J. Trop. Agric. Food Sci., 45: 37-50.
7. Olawale, A.K., O.M. David, A. Onasanya, A.O. Ajayi, R.T. Osuntoyinbo, O.O. Idris and O.J. Oje, 2017. Occurrence of antibiotic resistance and virulent factors in *Enterococcus faecalis* isolated from bush meat roasted and sold along road sides in Ekiti State. Curr. Res. Bacteriol., 10: 9-15.
8. Adzitey, F., P. Assoah-Peprah and G.A. Teye, 2019. Whole genome sequencing of *Escherichia coli* isolated from contaminated meat samples collected from the Northern region of Ghana revealed the presence of multidrug resistant genes. J. Global Antimicrob. Resist., 18: 179-182.
9. King, A., 2001. Recommendations for susceptibility tests on fastidious organisms and those requiring special handling. J. Antimicrob. Chemother., 48: 77-80.
10. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
11. CLSI., 2008. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Approved Guideline M45-A, Clinical and Laboratory Standards Institute, Wayne, PA., USA.
12. Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to indentify high-risk sources of fecal contamination of foods. Applied Environ. Microbiol., 46: 165-170.
13. Rishmawi, N., R. Ghneim, R. Kattan, R. Ghneim and M. Zoughbi *et al*., 2007. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. J. Clin. Microbiol., 45: 1278-1283.
14. Doern, G.V., 2000. Detection of selected fastidious bacteria. Clin. Infect. Dis., 30: 166-173.
15. OIE., 2018. African swine fever. World Organization for Animal Health, Paris, France. https://www.oie.int/en/animal-health-in-the-world/animal-diseases/african-swine-fever/
16. FAO., 1994. Specific Diseases of Pigs. In: Manual on Meat Inspection for Developing Countries, Herenda, D., P.G. Chambers, A. Ettriqui, P. Seneviratna and T.J.P. da Silva (Eds.). Chapter 4, Food and Agriculture Organization, Rome, Italy, ISBN-13: 9789251033043, pp: 193-244.
17. Adzitey, F., 2015. Antibiotic resistance of *Escherichia coli* isolated from beef and its related samples in Techiman Municipality of Ghana. Asian J. Anim. Sci., 9: 233-240.
18. Adzitey, F., S. Nafisah and A. Haruna, 2015. Antibiotic susceptibility of *Escherichia coli* isolated from some drinking water sources in Tamale Metropolis of Ghana. Curr. Res. Bacteriol., 8: 34-40.
19. Adzitey, F., 2018. Antibiotic resistance of *Escherichia coli* and *Salmonella enterica* isolated from cabbage and lettuce samples in Tamale metropolis of Ghana. Int. J. Food Contam., Vol. 5. 10.1186/s40550-018-0068-z.
20. McEwen, S.A. and P.J. Fedorka-Cray, 2002. Antimicrobial use and resistance in animals. Clin. Infect. Dis., 34: S93-S106.
21. Adzitey, F., C.K.S. Saba and A.T. Gabriel, 2016. Antibiotic susceptibility of *Escherichia coli* isolated from milk and hands of milkers in Nyankpala community of Ghana. Curr. Res. Dairy Sci., 8: 6-11.
22. Elkenany, R.M., 2018. Genetic characterization of enterotoxigenic strains of methicillin-resistant and susceptible *Staphylococcus aureus* recovered from bovine mastitis. Asian J. Biol. Sci., 11: 1-8.
23. Ayefoumi, A.C., D.H.A.K. Amewowor, I.K.A. Galyuon, E.P. Otwe, D.K.A. Asante, L. Yafetto and M.B. Adinortey, 2019. Molecular characterization of antibiotic resistance genes in *Escherichia coli* from clinical and environmental samples in Ghana. Asian J. Biol. Sci., 12: 356-363.
24. Abdel Azim, N.S., M.Y. Nofal, M.A. AlHarbi, M.I. Al-Zaban and A.M. Somily, 2019. Molecular-diversity, prevalence and antibiotic susceptibility of pathogenic *Klebsiella pneumoniae* under Saudi condition. Pak. J. Biol. Sci., 22: 174-179.