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Research Article

PHENOTYPIC CHARACTERISATION OF RESISTANCE PROFILES IN *PROTEUS VULGARIS* ISOLATED FROM PIG FAECES IN ASHANTI REGION, GHANA

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ABSTRACT
Proteus vulgaris are commensals and pathogens with little antibiotic resistance profiles characterisation in food animals. Their widespread distribution in humans, animals, birds and the environment makes them a potential threat to public health and food safety should they harbour resistance genes and virulence traits concurrently. To characterise the resistance profiles of *Proteus vulgaris* in food animals with exposure and non-exposure to antibiotics, the sensitivity of 54 (71.05%) porcine faecal isolates from 76 pig farms in Ashanti region, Ghana, to eight antibiotics were determined; one strain was taken per farm.
All the strains showed resistance to at least one antibiotic though strains from pigs exposed to antibiotics had higher resistance levels (43.42%) than those unexposed to antibiotics (27.63%)($p<0.0001$). Resistance to amoxicillin was higher in strains from pigs with no antibiotic exposure whiles resistance to streptomycin, the tetracyclines and sulphamethoxazole-trimethoprim were more pronounced in strains from pigs exposed to antibiotics. Resistance to antibiotics with less patronage among the farms were similar among all the isolates. Multidrug resistance was higher (50%) in strains from antibiotic-exposed pigs than that of strains from non-antibiotic exposed pigs (33.33%)($p<0.0001$).
Antibiotic use affects the ecology of commensal *Proteus vulgaris* and selects for multidrug resistant phenotypes; nevertheless, *P. vulgaris* has inherent resistance to amoxicillin, streptomycin and other antibiotics. Prudent antibiotic use is advised to reduce the annihilation of susceptible and non virulent strains which could compete with resistant and virulent strains to reduce the preponderance of resistant phenotypes that are a threat to public health.

Keywords: *Proteus vulgaris; Pigs; Ashanti Region; Antibiotic Resistance; Antibiotics; Pig Faecal Isolates*

INTRODUCTION
Proteus spp. have a wide distribution in nature, being found in soils, polluted waters, animals, birds and humans (Phillips, 1955; Senior and Leslie, 1986; Abbott, 2007). In humans, birds and other animals, they form part of the normal intestinal flora and are members of the *Enterobacteriaceae* (Public health agency of Canada, 2011). Nevertheless, they are also pathogenic in young people and opportunistic pathogens in very old humans under antibiotic treatments or hospitalised in long term care facilities (Coker *et al.,* 2000; Kim *et al.,* 2003). In humans they are known to cause urinary tract infections (UTIs) of severer fatalities than *Escherichia* coli (Senior and Leslie, 1986), urolithiasis, cystitis, acute pyeolonephritis, septicaemia, bacteraemia and wound infections (Coker *et al.,* 2000; Kim *et al.,* 2003; Abbot, 2007).
Proteeae have been associated with diseases in animals. *Proteus vulgaris* has been implicated in abortion and illness in new born horses, funiculitis following castration and hoof canker (Phillips, 1955). Though *P. Vulgaris* are found in pigs, their primary pathogenic role is doubtful (Andreev, 1940a; Andreev, 1940b); however, they are recognised as pathogens in turkey poults (Biester and Schwarte, 1952; Phillips, 1955). In several cases, *Proteus* bacilli have been isolated from tissues of cattle showing signs of enteritis or metritis *ante mortem* and in calves, they have been incriminated in pneumo-enteritis (Phillips, 1955). Among the *Proteeae* tribe, *P. vulgaris* and *P. mirabilis* are commonly encountered (Phillips, 1955; Senior and Leslie, 1986). *P. vulgaris* and *P. mirabilis* are common among animals, especially cattle and pigs, and

in humans respectively (Phillips, 1955; Senior and Leslie, 1986).*P. mirabilis* is the most virulent and commonly isolated *Proteeae* in UTI cases involving this tribe (Senior and Leslie, 1986), having a predilection for the upper urinary tract where it damages the renal tubular epithelium (Eden *et al.,* 1980) and forms renal stones through urease reactions that breaks down urea and makes the urine alkaline (Brauda *et al.,* 1960; Griffith *et al.,* 1973; Senior *et al.,* 1980; Senior, 1983).

Proteus spp. has been associated with both community-acquired infections (4-6% of proteus infections) and nosocomial infections (3-6% of proteus infections) in Europe and North America (Abbott, 2007). Coupled with their wide distribution in the environment and in humans, animals and birds, they have the potential of affecting a wider domain of hosts should there be an outbreak of resistant virulent strains. As commensals, they can also serve as reservoirs of resistance genes which they can pass on to pathogens of the same *Proteeae* tribe, especially mirabilis or of other species (Nijsten *et al.,* 1994). *Proteeae* are generally subsceptible to broad spectrum aminoglycosides, cephalosporins and imipenem (Abbott, 2007) and *P. Vulgaris* is reportedly resistant to amoxicillin, ampicillin, cefuroxime, cefoperazone and cefazolin (Coker *et al.,* 2000). However, Isolated reports have recently been describing increasing trends in resistance and even pan drug resistance among *Proteeae* to the β-lactams including carbapenems (Kumarasamy *et al.,* 2010).

Interestingly however, *Proteeae* are relatively less studied and characterised in terms of their resistance compared to other commensals and pathogens. Valuable data on this tribe are limited and mostly out-dated. To characterise the antibiotic resistance profiles of *P. vulgaris,* which is the commonest *Proteeae* in cattle and pigs (Phillips, 1955; Senior and Leslie, 1986), seventy six pig farms in the Ashanti region, Ghana were studied. The effect of antibiotics on resistance and ecology of *P.* Vulgaris were determined and compared with strains with little or no exposure to antibiotics. The study aimed to characterise the resistance profiles of these species in food animals as an assessment of their potential role in both harbouring and spreading resistance genes and causing resistant infections in consumers, farmers, farming communities and abattoir workers through contaminated food animal carcasses and close contact respectively (Levy, 1978; Ojeniyi, 1989; Nijsten *et al.,* 1994; Van den Bogaard *et al.,* 2001).

MATERIALS AND METHODS

Method

The study was conducted among 76 pig farms located in 10 towns in five districts within the Ashanti region, Ghana from January to December 2012. The selection of the farms were carefully done after an initial survey which collected the antibiotic usage patterns data from farms within each district in the Ashanti region. This survey was done by a principal investigator in the local language, *Twi,* to collect information on the types and frequency of antibiotic usage among the farms. The data generated was used to group the farms into two classes: those that had not used antibiotics on all or some of their animals within the past one year (NA) and those that were frequent users of antibiotics (AU).

Consequently, 38 NA and 38 AU farms were selected. Fresh faeces (one sample) were collected from each farm within each category; within NA farms, faeces were obtained from animals not exposed to antibiotics. Faecal samples were immediately transferred on ice to the laboratory for microbiological analysis. Suspensions of each faecal sample were prepared in 0.9% saline with 20% glycerol and stored at -20°C until analysed.

Stored samples were thawed before microbiological analysis. Pure *Proteus vulgaris* colonies were isolated and detected from the samples according to protocols described by Peer booms and peers (1985). An isolate was taken per sample. A 0.5 McFarland's standard, using 0.9% saline, was prepared from each pure isolate and used for antibiotic sensitivity testing using CLSI (2012) standards and protocols. Antibiotic sensitivity testing discs (Gentamicin (Gen)-10µg, Streptomycin-10µg, Norfloxacin (Nor)-10µg, Ciprofloxacin (Cip)-5µg, Tetracycline (Tet)-30µg, Doxycycline (Dox)-30µg, Amoxycillin (Amo)-10µg and Sulphamethoxazole/Trimethoprim (SXT)-25µg) from Oxoid (Oxoid, Basingstoke, UK) and a semi-automated multi disc dispenser (Oxoid, Basingstoke, UK) were used to determine the sensitivities of the *Proteus vulgaris* isolates according to described standards (CLSI, 2012). Positive controls were set up

for every batch of plates tested using *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. All tests were carried out in triplicates to ensure reproducibility.

The zones of inhibition produced by the antibiotics were measured thrice and the average was compared with the CLSI (2012) tables to determine the susceptibility levels of the various bacterial isolates. Strains with resistance to more than two antibiotics were classified as multi drug resistant (MDR).

Data Analysis

The number and percentages of resistant isolates to amoxicillin, ciprofloxacin, norfloxacin, gentamicin, streptomycin, tetracycline, doxycycline and Sulphamethoxazole-trimethoprim were analysed with Microsoft Excel© 2010 (Microsoft Corporation, Microsoft office package, 2010, USA). Susceptible and intermediate resistant strains were left out of the analysis.

Ethical Approval and Informed Consent

Ethical exemption and study approval were obtained from the Faculty of Pharmacy of the Kwame Nkrumah University of Science and Technology. Informed consent was not required though the approval of the regional and district Veterinary offices and the farmers were obtained before faecal collection began.

RESULTS AND DISCUSSION

Results

A total of 76 pig faecal samples, one sample from a farm, were used for the study. However, 54 (71.05%) *P. vulgaris* strains, one per sample, were obtained from this total: 21 (27.63%) and 33 (43.42%) were obtained from NA and AU farms respectively. All the isolated *P. vulgaris* strains, irrespective of farm category and antibiotic usage pattern of the farm, were resistant to one or more of the antibiotic(s) tested (table 1) with distinct variations ($p<0.0001$). Resistance to amoxicillin and streptomycin was common in NA farms with resistance to amoxicillin in NA farms being twice that found in AU farms (figure 1 and table 1). Resistance to antibiotics with lower patronage among the farms (ciprofloxacin, norfloxacin and gentamicin) were fairly similar between both classes (AU and NA) (figure 1 and table 1). Resistance to antibiotics mostly patronised by the farms (the tetracyclines, sulphadimidine and streptomycin) were highest among isolates from AU farms (figure 1 and table 1) ($p<0.0001$).

Table 1: Antibiotic resistance profiles of *Proteus vulgaris* isolated from pigs exposed (AU) and unexposed (NA) to antibiotics.

	Non-antibiotic using farms (NA) (n=21)		**Antibiotic-using farms (AU) (n=33)**	
Antibiotic	**Number of resistant strains (%)**	**Percentage (%) of resistant strains per all isolates (n=54)**	**Number of resistant strains (%)**	**Percentage of resistant strains per all isolates (n=54)**
Amoxicillin	18 (85.71)	33.33	9 (27.27)	16.67
Ciprofloxacin	3 (14.29)	5.56	3 (9.09)	5.56
Norfloxacin	3 (14.29)	5.56	0 (0)	0
Gentamicin	6 (28.57)	11.11	3 (9.09)	5.56
Streptomycin	15 (71.43)	27.78	18 (54.55)	33.33
Tetracycline	3 (14.29)	5.56	18 (54.55)	33.33
Doxycycline	9 (42.86)	16.67	12 (36.36)	22.22
Sulphamethoxazole-trimethoprim	6 (28.57)	11.11	24 (72.72)	44.44

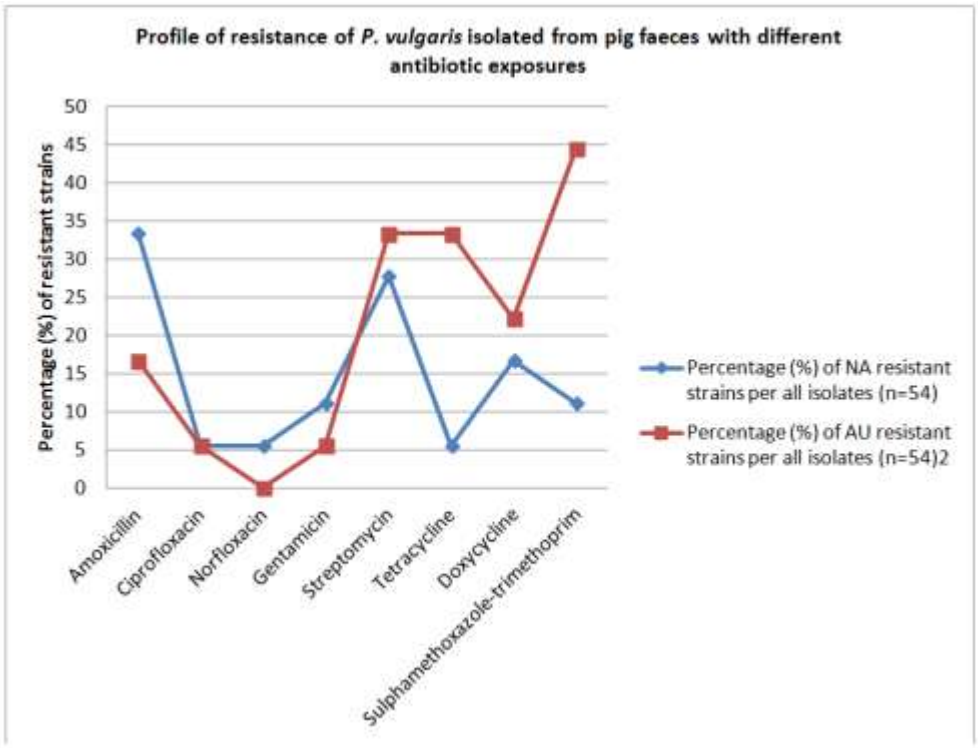

Figure 1: levels of resistance (%) of *Proteus vulgaris* strains isolated from antibiotic using (AU) and non-antibiotic using (NA) farms to tested antibiotics

A comparison of the percentage resistance levels of *Proteus vulgaris* isolates from two farm groups, antibiotic using farms (AU) and non antibiotic using farms (NA), are shown in figure 1. It can be seen that overall, isolates from AU farms have a higher level of resistance than NA farms ($p<0.0001$).

Table 2: Spectrum of antibiotics to which P. vulgaris isolates showed multidrug resistance (MDR).

	Non-antibiotic using farms (NA) (n=21)		**Antibiotic-using farms (AU) (n=33)**	
Antibiotics	**Number of MDR strains (%)**	**Percentage (%) of MDR strains per all isolates (n=54)**	**Number of MDR strains (%)**	**Percentage of resistant strains per all isolates (n=54)**
Amo and Stp	12 (57.14)	22.22	0	0
Amo and Dox	9 (42.86)	16.67	3 (9.09)	5.56
Amo, Cip and Nor	3 (14.29)	5.56	0	0
Amo, Cip, Nor, Stp and STX	3 (14.29)	5.56	0	0
Amo, Stp, Tet and Dox	3 (14.29)	5.56	0	0
Amo, Gen and STX	3 (14.29)	5.56	0	0
Amo, Gen, Stp	3 (14.29)	5.56	0	0

and Dox				
Stp, Dox, Tet and STX	0	0	6 (18.18)	11.11
Stp, Dox and STX	0	0	3 (9.09)	5.56
Amo, Tet, STX	0	0	6 (18.18)	11.11
Stp and STX	0	0	12 (36.36)	22.22
Tet and Dox	0	0	9 (27.27)	16.67
Amo and Tet	3 (14.29)	5.56	9 (27.27)	16.67
Gen, Stp, Tet, Dox and STX	0	0	3 (9.09)	5.56
Cip and Stp	0	0	3	5.56
Tet and STX	0	0	12	22.22
Stp and Tet	0	0	9	16.67

This is especially obvious from streptomycin to sulphamethoxazole-trimethoprim, antibiotics in common use among the farmers. Resistance in isolates from NA farms to antibiotics not used in the farms (norfloxacin, ciprofloxacin and ampicillin) are equal or slightly higher than that of isolates from AU farms.

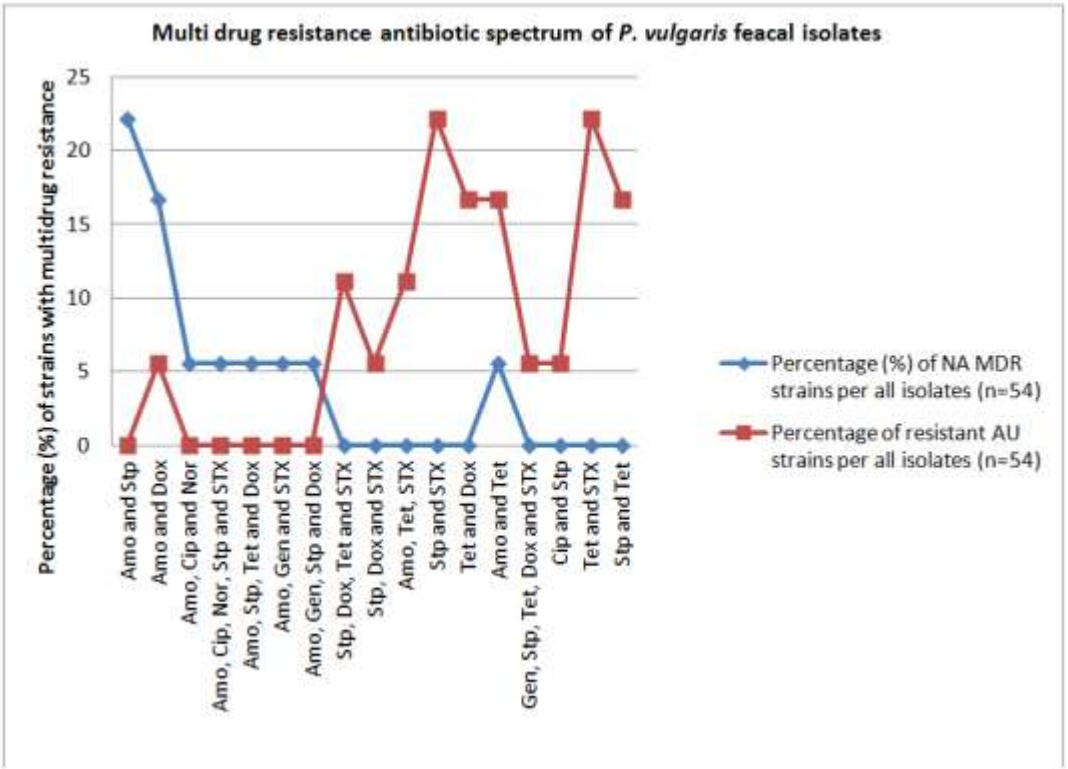

Figure 2: Antibiotic resistance spectrum of multidrug resistant *P. vulgaris* faecal isolates exposed or unexposed to antibiotics

Moreover, multidrug resistance was observed in strains from both sides of the divide: 85.71% (33.33% of all isolates; n=54) of the NA isolates and 81.81% (50% of all isolates; n=54) of AU isolates were multidrug resistant (MDR) ($p<0.0001$). Consequently, multidrug resistance was more pronounced among isolates from AU farms than those from NA farms (figure 2 and table 2). Table 2 shows the spectrum of antibiotics to which isolates from both groups were resistant.

The spectrum of antibiotics to which the isolated *P. vulgaris* strains showed multi drug resistance are represented in figure 2 above. It can be observed that antibiotics to which isolates from AU farms show multidrug resistance (MDR) to are vastly different and opposite to that of isolates from NA farms. The combinations of antibiotics to which the isolates from AU farms show MDR are those which are commonly used by the pig farmers. Overall, MDR strains were more common in AU farms than NA farms ($p<0.0001$).

Discussion

Though *Proteus vulgaris* are common intestinal denizens less associated with urinary tract infections vis-à-vis *Proteus mirabilis* and *Escherichia coli*, they are known be pathogenic among animals, younger people and the elderly (Phillips, 1955; Senior and Leslie, 1986; Kim *et al.*, 2003). Irrespective of their wider distribution in nature and presence in pigs and cattle (Phillips, 1955), their potential role as reservoirs of resistance and possible public health threat has been largely understudied. The resistance profiles of *P. vulgaris* isolates with different exposure to antibiotics are herein studied.

The presence of resistant and MDR strains in NA farms is suggestive of inherent resistance in *Proteus vulgaris* (table 2 and figure 2). Though it may be argued that the pigs from NA farms were not kept in closed confinement and monitored to ensure the total absence of antibiotic, resistant bacteria and resistance genes exposure, the similarities and general resistance patterns observed in all the isolates from the NA farms vis-à-vis the AU farms seems to override and normalize the interference of these nuances. Consequently, the inherent resistance of these strains, especially to amoxicillin and streptomycin, cannot be gainsaid; however, further molecular studies would be necessary to ascertain the means of resistance acquisition; either by mutation, horizontal or vertical gene transfer.

The proportion of *P. vulgaris* isolates obtained from the NA (27.63%) and AU farms (43.42%) suggests a possible antibiotic effect on the intestinal microbial ecology. The use of antibiotics in AU farms possibly annihilated several competitive but susceptible bacterial species, allowing the resistant *Proteus vulgaris* freedom to multiply and occupy more space; the absence of such a phenomenon in the intestines of pigs not exposed to antibiotics makes it impossible for *Proteus vulgaris* to multiply in the face of competition from other bacterial species; hence their lower isolation success. On the other hand, the lower levels of resistance to amoxicillin observed in AU farms could be due to the annihilation of amoxicillin-resistant strains which could be susceptible to the antibiotics used among AU farms. Such a condition would enable the relative proliferation of resistant strains in the intestines of antibiotic-exposed pigs. Therefore, the combinations of antibiotics to which the multidrug resistant strains were resistant reflects this selection pressure (table 2 and figure 2)

The relatively minor differences observed in the resistance levels (table 1 and figure 1) against antibiotics with lower patronisation among the farms (ciprofloxacin, norfloxacin and gentamicin) lend support to the above arguments. Because *P. vulgaris* populations from both farm groups have little or no exposure to these antibiotics, the selection of resistant strains and the disturbance of the microbial ecology is not pronounced; albeit not totally absent.

The concomitant acquisition of resistance to antibiotics of the same class as those used on the farms (cross resistance) was observed in the tetracyclines (tetracycline and doxycycline) and sulphamethoxazole-trimethoprim. The main tetracyclines used by the farmers were oxytetracycline (data not shown) whiles sulphadimidine was the main sulphonamide used. However, the higher resistances expressed towards tetracycline and doxycycline and sulphamethoxazole-trimethoprim, antibiotics not specifically used in larger proportions by the farmers, suggest the possibility of cross resistance. That these trends were not seen among strains from NA farms cancels out the possibility of inherent resistance.

These resistance patterns agree with those earlier reported for *P. Vulgaris* (Coker *et al.*, 2000; Abbott, 2007) and adds to the call to stem the abuse of antibiotics in veterinary and clinical medicine in the bud (Teuber, 2001). The effects of antibiotics on the microbial ecology and resistance observed demonstrates the need to adopt husbandry practices that reduces the use of antibiotics and radical policies that bans several antibiotics from veterinary medicine. The importance of such policies in ameliorating the effect of antibiotics has been well documented in Denmark, Sweden and Norway after the ban of antibiotic growth promoters in food animal production (WHO, 2011).

Conclusion

Antibiotic use affects the ecology of commensals like *P. vulgaris* in animals and provides an advantage to resistant strains to proliferate. *P. vulgaris* expresses inherent resistance especially to amoxicillin and streptomycin and is a reservoir of resistance genes selected by antibiotic use. Consequently, it could spread these genes to pathogens, resulting in fatal infections to consumers of food animals, farmers and abattoir workers. The need to restrict antibiotic use in food animal production is recommended in reducing the selection of spread of resistant phenotypes.

Competing Interests

The author has no competing interests to declare. The sponsors had no role or whatsoever in the preparation of the manuscript, data collection and analysis and decision to publish.

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