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Potential Effects of Antibiotics on Selected Bacteria Isolated from Cow's Rumen

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Abstract: Action and reaction of antibiotics on ruminal bacteria cannot be predicted. This study therefore preferentially evaluated the *in – vitro* effects of antibiotics on the ruminal bacteria of cow from the Central Abattoir Complex, Agege, Lagos State, Nigeria. Rumen samples were cultured, isolated, characterized and identified based on standard microbiological protocols. Out of the 75 ruminal samples collected, a total of 125 aerobic ruminal bacteria were isolated. Based on interest, the following bacteria were isolated; *Streptococcus* spp., *Staphylococcus aureus*, *Pseudomonas* spp. and *Salmonella* spp. *Streptococcus* spp. had the highest occurrence (33%), followed by *Staphylococcus aureus* (24%). Among the antibiotics, Ciprofloxacin was observed as the best in comparison to Gentamycin, Pefloxacin, Erythromycin, Zinacef and Nalidixic acid that were also found to be effective. The isolates exhibited gross resistance to Ampicillin and Ampiclox. This investigation revealed that ruminal bacteria from cow can simultaneously exhibit both sensitivity and resistance to antibiotics.

Key words: Antibiotics · Isolates · Bacteria · Susceptibility · Resistance · Ruminant · Cow

INTRODUCTION

A wide variety of antibiotics are routinely added to animal feed in sub-therapeutic doses for growth promotion of animals produced for human consumption [1, 2]. They also have positive effects on lactation performance, as well as decreased incidence and severity of disease including reduction in mortality [3].

In Nigeria, several antibiotics have been used at sub-therapeutic levels in ruminant production systems. Specifically, in Ekiti State between the year 2002 and 2004, there was a gradual increase in the use of various antibiotics including quinolones, gentamycin, neomycin, tylosin and chloramphenicol [4].

Feeding antibiotics to livestock might lead to changes in the commensal bacteria in the gastrointestinal tract of animals fed with such antibiotics [5]. Ciprofloxacin, erythromycin, amikacin, vancomycin, piperacillin, cefotaxime, streptomycin and ciprofloxacin are among the antibiotics that have demonstrated sensitivity against ruminal bacteria [6, 7].

Apart from sensitivity, Fisher and Scott [8] suggested that resistance is more likely to appear when physicians and veterinarians misdiagnose infections and improperly administer antibiotics. Similarly, misuse and overuse of antibiotics can play a critical role in the development of resistance [9, 10]. There are numerous reports on isolation of antibiotic-resistant bacteria from livestock. Among the livestock, attention has been placed on the use of antibiotics for poultry and swine [11] while that of cow especially on their ruminal bacteria has been relegated to the background. Knowing fully well that antibiotics can develop significant effects against ruminal bacteria [6], therefore, it is expedient to focus on cow known to be mostly consumed by humans [12-15] and can as well be a good host to either sensitive or resistant ruminal bacterial strain [6, 7]. Based on this understanding, preliminary investigation was carried out on the in – vitro effects of antibiotics on bacteria isolated from cow's rumen from the Central Abattoir Complex, Agege, Lagos State, Nigeria.

MATERIALS AND METHODS

Collection of Samples: Rumen of slaughtered cows was periodically collected from the Central Abattoir Complex located at Oke-Oba, Agege Local Government Area, Yaba in Lagos State, Nigeria. The samples were collected on

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weekly basis for five consecutive weeks. Each week, 15 sterile swab sticks were used to collect the rumen samples (both solid and liquid materials). Overall, 75 cows were sampled irrespective of their species, age and gender. After each collection, the labeled samples were immediately and aseptically transported to the Microbiology Department of the Nigeria Institute of Medical Research, Yaba, in Lagos State for bacteriological analysis.

Isolation and identification of bacteria Specifically, the following media; Nutrient Agar (NA), Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA), Sorbitol MacConkey Agar (SMA) and Kligler Iron Agar (KIA) were prepared according to the manufacturer's specifications and instructions and were used to determine selected bacteria contents in the rumen samples. Using streak plate method [16, 17], all the plates were inoculated in duplicate and incubated aerobically at 37 ± 2°C for 24 hours. Attention was placed only on aerobic bacteria of interest. Distinct colonies from each of the isolates were obtained and sub cultured onto a freshly prepared NA followed by incubation at 37 ± 2°C for 24 hours. This step was repeated severally until pure cultures were obtained for each isolate. Pure cultures of the bacterial isolates were maintained on NA slants and were stored at 4°C. The bacterial isolates were identified based on their morphological and biochemical characteristics using Bergey's Manual of Determinative Bacteriology [19].

Antibiotic Susceptibility Test: Prior antibiotic susceptibility test, McFarland standard corresponding to 0.5 was prepared [20, 21]. Turbidity was confirmed to have optical density (OD) of 0.08 - 0.10 at 625nm using photo-electric colorimeter. The antibiotic susceptibility of the isolates was performed using selected Gram specific antibiotics (Oxoid, Basingstoke, Hampshire, England) which included Pefloxacin (10ug), Gentamycin (10ug), Ampicillin (30ug), Ampiclox (30ug), Zinacef (20ug), Amoxicillin (30ug), Ciprofloxacin (10ug), Streptomycin (25ug), Septrin (10ug), Erythromycin (10ug) and Nalidixic acid (20ug). Using a sterile inoculating loop, the distinct colony of each isolate was emulsified in 3 – 4 ml of sterile physiological saline and turbidity of the bacterial suspension was matched to the turbidity of the standard. The bacterial suspension was swabbed with sterile cotton swab evenly on Mueller Hinton agar in Petri - dishes by rotating plate at approximately 60°. The multi - antibiotic discs (Oxoid, Basingstoke, Hampshire, England) was placed aseptically onto inoculated three replicated plates with respect to each isolate using sterile forceps. This was incubated aerobically at 37 ± 2°C for 24 - 48 hours [22, 23]. Diameters of the zones of inhibition were measured with a ruler and recorded in millimeter (mm). The interpretation of the results was done using interpretative chart according to CLSI [24]. Based on the interpretation, the bacterial isolates were classified either as susceptible or resistance using 50% as the breaking point.

RESULTS

Occurrence of Ruminal Bacteria: Out of the 75 ruminal samples evaluated, a total of 125 aerobic ruminal bacteria were isolated. Gram reaction shows that 71 of the isolates were Gram positive while 54 were Gram negative. Bergey's Manual of Determinative Bacteriology reveals isolates identity as *Streptococcus* spp., *Staphylococcus aureus*, *Pseudomonas* spp. and *Salmonella* spp. *Streptococcus* spp. had the highest occurrence (33%), followed by *Staphylococcus aureus* (24%) while *Salmonella* spp. (20%) had the least (Fig. 1).

Sensitivity of Antibiotics on Bacterial Isolates: The phenotypic resistance or sensitivity of the rumen bacteria varied with respects to the antibiotics used in this study. Specifically, all the isolates exhibited gross resistance (Table 1) to Ampicillin (100%) and Ampiclox (100%). Ciprofloxacin was found to be generally effective on all the Genus isolates with average effectiveness of 77%. In addition, Gentamycin, Pefloxacin, Ciprofloxacin, Erythromycin, Zinacef and Nalidixic acid were also observed to be effective. They demonstrated high sensitivity against the ruminal bacteria, though variation was observed with respect to different isolates (Table 1).

Furthermore, observation shows that *Staphylococcus aureus* was resistance to virtually all the antibioitcs with exception of Gentamycin and Ciprofloxacin that had 93% and 60% respectively. Pefloxacin (97%), Ciprofloxacin (72%) and Nalidixic acid (69%) were highly effective on *Pseudomonas* spp. while other antibiotics were extremely less sensitive. Ciprofloxacin (78%) and Streptomycin (98%) showed remarkable sensitivity on *Streptococcus* spp. in comparison with Pefloxacin (54%), Zinacef (54%) and Erythromycin (54%) that exhibited moderate effectiveness on it. However, other antibiotics were relatively uneffectiveness on *Streptococcus* spp. Similarly, apart from Gentamycin (72%), Pefloxacin (88%), Ciprofloxacin (96%) and Nalidixic acid (80%), other antibiotics were regarded as less effective on *Salmonella* spp.

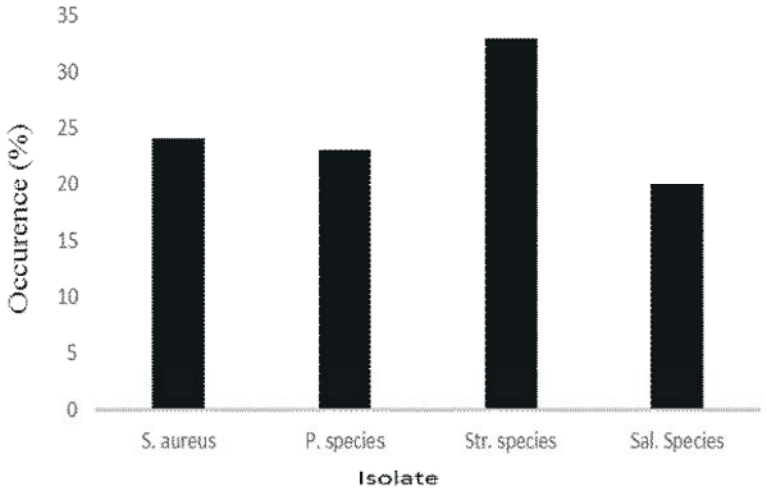

Fig. 1: Occurrence (%) of bacterial isolates. *S. aureus* = *Staphylococcus aureus*, *P. species* = *Pseudomonas* spp., *Str. Species* = *Streptococcus* spp., *Sal. Species* = *Salmonella* spp.

Table 1: Sensitivity profiles of antibiotics on bacterial isolates

	Isolate					
Antibiotic	Antibiotic Status	*Staphylococcus aureus* (%)	*Pseudomonas* spp. (%)	*Streptococcus* spp. (%)	*Salmonella* spp. (%)	Average Susceptibility (%)
Ampicillin	S	0	0	0	0	0
	R	100	100	100	100	100
Gentamicin	S	93	34	44	72	61
	R	7	66	56	28	39
Pefloxacin	S	10	97	54	88	62
	R	90	3	46	12	38
Ampiclox	S	0	0	0	0	0
	R	100	100	100	100	100
Amoxicillin	S	17	0	12	7	9
	R	83	100	88	93	91
Erythromycin	S	0	24	54	34	28
	R	100	76	46	66	72
Ciprofloxacin	S	60	72	78	96	77
	R	40	28	22	4	23
Streptomycin	S	0	41	98	40	45
	R	100	59	2	60	55
Septrin	S	0	10	0	12	6
	R	100	90	100	88	93
Zinacef	S	33	38	54	48	43
	R	67	62	46	52	57
Nalidixic acid	S	40	69	5	80	49
	R	60	31	95	20	51

S = Sensitive,
R = Resistance

Generally, percentage of sensitive isolates varied with respect to each antibiotic. High numbers of sensitive *Streptococcus* spp., *Salmonella* spp. and *Pseudomonas* spp. were observed against Ciprofloxacin and partly Streptomycin. The potential effect of Amoxicillin on the isolates was relatively low in comparison to that of Gentamycin, Erythromycin, Pefloxacin and Streptomycin. *Staphylococcus aureus* exhibited high level of resistance to Streptomycin. Ironically, the same Streptomycin was found to be effective on *Streptococcus* spp. and *Salmonella* spp. Further observation showed that *Streptococcus* spp. discouraged the effectiveness of Nalidixic acid and Gentamycin (Table 1).

DISCUSSION

Cow's rumen remains one of the important reservoirs of abundant microbiota (comprising ~ 10^{10} cells per ml of contents) that converts indigestible plant biomass into compounds that can be used by cow [25]. A substantial low level of aerobic bacteria was recorded from the cow's rumen. Aerobic bacteria are generally few in the rumen of animals in comparison to anaerobes that are much [26]. *Streptococcus* spp. (33%), *Pseudomonas* spp. (23%), *Salmonella* spp. (20%) and *Staphylococcus aureus* (24%) varied in their occurrence [26-28]. This variation in their occurrence may be attributed to different types of antibiotics used by the farmers.

Majority of the isolates varied in their antibiotic sensitivity and resistance [28, 29]. However, observation revealed that the bacterial isolates were more sensitive to a substantial number of antibiotics evaluated. This is an indication that sub-therapeutic antibiotics use in ruminant feeding to optimize rumen fermentation may as well increase the inhibition of ruminal bacterial populations [6]. Specifically, *Streptococcus* spp. was more sensitive to Zinacef (54%), Erythromycin (54%), Streptomycin (98%), Ciprofloxacin (78%) and Pefloxacin (54%). This suggests that whenever these antibiotics are used in feed or applied to treat cow, there may be possibility of reducing the load of *Streptococcus* spp. Similarly, *Salmonella* spp. was extremely sensitive to 40% of all the antibiotics. This corroborated the work of Salehi *et al*. [29] who also observed the effects of Ciprofloxacin, Nalidixic acid and Streptomycin on different *Salmonella* serogroup isolated from intestine and liver of poultry. Anyanwu *et al*. [30] also confirmed the effects of Ciprofloxacin (96%) against *Salmonella* spp. isolated from day - old chicks in Nigeria.

The sensitivity of Ciprofloxacin was noticeable on *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus* spp. (77%). This is in line with the understanding of Salem *et al*. [7] who also documented similar observation using Gentamycin, Ciprofloxacin and Streptomycin against the mutant ruminal bacterial isolates from Sheep, Cattle and Buffalo. Another study from Marama et al. [31] confirmed the potency of Gentamycin on *Staphylococcus aureus* isolated from 384 lactating Holstein cross breed, jersey breed and zebu cows in Holeta Area, Western Ethiopia. This is an indication that the ruminal bacteria causing diseases in Nigerian cow can be treated with few antibiotics among which include Gentamycin and Ciprofloxacin. This suggest that when meat handlers get infected by these bacteria, treatment can commence without the fear that the isolates will resist the drug used. However, abuse or misuse of these antibiotics can be dangerous to both animal and human.

All the isolates demonstrated gross resistance (100%) to Ampicillin and Ampiclox. This may be attributed to long term use of Ampicillin and Ampiclox for cow in Nigeria [32]. Furthermore, resistance established in this manner may exhibit a high degree of stability, allowing resistant bacteria to compete with antibiotic-susceptible bacteria even in the absence of the antibiotics [33]. Feeding antibiotics at low, that is, sub-therapeutic, levels can accelerate development of antibiotic resistance, because more bacteria are likely to survive the challenge and also because the period of exposure of the microbial population to the antibiotic is prolonged [28].

The level of resistance demonstrated by *Staphylococcus aureus* against Streptomycin (100%), Erythromycin (100%), Streptomycin (100%), Septrin (100%) and Pefloxacin (90%) and that of *Streptococcus* spp. against Nalidixic acid (95%) is alarming. This is expected because *Staphylococcus aureus* can display resistance to several relevant antibiotics making its eradication difficult [34] and this has become a challenge to diary industry. Thus, continuous surveillance and monitoring of its prevalence in diary animals and its antibiotic resistance patterns have a paramount public health implications [31]. Similarly, apart from Ampicillin and Ampiclox, the resistance of *Pseudomonas* spp. to Amoxicillin (100%) and Septrin (90%) were on the high side [35]. This may be attributed to misuse and abuse of antibiotics [10, 9 & 35] to cow. Thus, using an antibiotic against bacteria for which it was not designed for will not only fail to control the disease, but will also increase the likelihood that other non-target bacteria will develop resistance [28]. In addition, the isolated bacteria may have evolved several antibiotic resistance mechanisms of exchanging genetic material [36]. It is obvious that bacteria are masters at developing antibiotic resistance. Given their immense evolutionary capacity, it appears that repetitive exposure of bacteria to any particular antibiotic will inevitably result in the development of some degree of resistance. Moreso, there is increasing evidence that antimicrobial use in animals specifically cow serve as selection pressure for resistant food-borne pathogen development that may be transmitted to humans as food contaminants and become life-threatening [37]. Therefore, the true concern is not whether the use of antibiotics in disease management will lead to the development of resistance, but whether those bacteria that do develop

resistance can pose a risk to human health [28]. Generally, the effects of antibiotics on ruminal bacteria of cow cannot be underestimated. Therefore, there is need to pay more attention to how these antibiotics will not implicate the public health.

CONCLUSIONS

Streptococcus spp. occurred more than other isolates from cow's rumen. Phenotypic susceptibility test uncovered both resistant and sensitive isolates. All the isolates exhibited gross resistance to Ampicillin and Ampiclox. Fortunately, Ciprofloxacin was outstanding among the antibiotics evaluated, demonstrating high potential effects on all the isolates. However, the fate of Ciprofloxacin is uncertain and resistance cannot be predicted due to the magnanimous power of bacteria against antibiotics. Generally, the use of these antibiotics in a sub-therapeutic dosage to promote growth and productivity of cow should be re-considered in-view to prevent future public health hazards. Therefore, sustainable, environmental and health friendly strategies towards the use of these antibiotics should be engaged.

Conflict of Interests: The authors have not declared any conflict of interests.

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