

Prevalence of *Corynebacterium* species among Slaughtered Ruminants in Makurdi, Nigeria: A Preliminary Study

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ABSTRACT

Non-diphtheritic *Corynebacteria* have in recent times been increasingly implicated as the causative agents of various infections in humans and animals. They have also been shown to be an emerging group of multidrug-resistant bacteria. In the present study, we carried out a preliminary investigation to assess the prevalence and antimicrobial susceptibility profile of species of *corynebacteria* among slaughtered cattle, goats and sheep. Nasal swabs from 207 ruminants (101 goats, 91 cattle, and 15 sheep) were processed for isolation and identification of *corynebacteria* using standard microbiological procedures. Antibigram of the isolates was also determined using the Kirby-Bauer disc diffusion technique. Twenty-three isolates (11.1%) distributed into six species comprising *Corynebacterium xerosis* (n=8), *C. amycolatum* (n=5), *C. mycetoides* (n=3), *C. stationis* (n=2), *C. striatum* (n=1) and *C. efficiens* (n=1) were recovered. The *Corynebacterium* isolates displayed high rates of resistance (31.6 – 100%) to all the antibiotics tested with multidrug resistance observed in 78.9% (15/23) of the isolates tested. Coagulase-production was also observed among 8 (34.8%) of the isolates. Our findings highlight the role of slaughtered cattle and small ruminants as potential reservoirs of multidrug resistant and zoonotic non-diphtheritic *corynebacteria* and thus a need for increased surveillance and characterization of this bacteria group among animals.

Keywords: *Corynebacteria*, infections, isolates, resistance, ruminants.

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I. INTRODUCTION

There are over 100 species of *Corynebacterium* isolated from humans, animals, and environmental sources [1], [2]. *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis* are the most important zoonotic and toxigenic species associated with different infectious processes in humans [3], [4]. Diphtheria toxin and/or phospholipase D are potent exotoxins produced by these species.

Non-diphtheritic *Corynebacterium* species associated with animal diseases include *Corynebacterium amycolatum*, *Corynebacterium aquilae*, *Corynebacterium auriscanis*, *Corynebacterium bovis*, *Corynebacterium camporealensis*, *Corynebacterium canis* and *Corynebacterium xerosis* [1],

[3]-[7]. Reference [5] was the first to report the isolation and identification of *Corynebacterium xerosis* from animal clinical specimens in Spain. Similarly, Reference [6] reported for the first time the isolation and molecular characterization of *Corynebacterium xerosis* from a clinical sample of an ovine cutaneous abscess in Mexico. Strains of *Corynebacterium bovis* and *Corynebacterium amycolatum* were respectively isolated from milk samples from clinical bovine mastitis and sub-clinically infected bovine quarters [7]. In a similar study, Reference [1] detected and identified, among others, *Corynebacterium xerosis* in raw milk samples from dairy farms in Germany.

Many recent studies have implicated non-diphtheritic *Corynebacteria* as the causative agents of various infections in humans. *Corynebacterium amycolatum* is the most frequently isolated diphtheroids [8]. *Corynebacterium*

amycolatum has been isolated from cases of bacteraemia, endocarditis, acute/chronic complicated skin and soft tissue infection in humans [2]. Twelve cases of ear infection with *Corynebacterium amycolatum* were reported by Reference [8]. Reference [9] reported a novel case of corneal ulcer in a 72-year-old patient with diabetes caused by *Corynebacterium amycolatum*. Similarly, *Corynebacterium striatum*, though considered contaminants, have been associated with infection, bacteraemia, and endocarditis [10]. This species can be considered an emerging pathogen for immune-compromised individuals as there are reports of cases of infection with *Corynebacterium striatum* in these patients [2], [11]–[13].

Recent reports have shown that some of the non-diphtheritic corynebacteria are emerging multidrug resistant bacteria. References [2] and [14] respectively reported that 86% and 72% of *Corynebacterium striatum* isolates in their studies were multidrug resistant. Reference [2] also reported the display of multidrug-resistance phenotypes to β -lactams, macrolides, clindamycin, aminoglycosides, quinolones, and rifampicin by all the isolates of *Corynebacterium amycolatum* in their study.

Despite the fact that recent studies have shown that the number of opportunistic infections caused by non-diphtheritic corynebacteria is on the rise in humans, especially in immunocompromised patients, and the emerging multidrug resistant corynebacteria, there is dearth of information about these bacteria in Benue State and in Nigeria generally. Documented reports on the roles of livestock in harbouring and disseminating these different zoonotic species of non-diphtheritic corynebacteria are lacking. This present study was, therefore, conducted to assess the prevalence and antimicrobial susceptibility profile of species of corynebacteria isolated from cattle, goats and sheep.

II. MATERIAL AND METHODS

A. Sample Collection and Processing

Using sterile plastic handle swabs, nasal swabs were collected from ruminants immediately after slaughter at Wurukum Abattoir in Makurdi, Benue State for a period of one month. A total of 207 samples comprising 101 goats, 91 cattle and 15 sheep were collected. The swabs were transported to the microbiology laboratory for *Corynebacterium* isolation within 4 hours of collection.

Each swab was inoculated directly onto Holye's tellurite agar supplemented with 5% sheep blood and incubated at 37 °C for 24–48 hours. After the period of incubation, a presumptive *Corynebacterium* colony that appeared brown to black, round and smooth were picked from each of the selective agar plate and subcultured on nutrient agar to obtain pure isolates for microscopy. Colonies that appeared as pleomorphic rods (straight or curve) with tapered and club-shaped ends, arranged singly and/or in pairs, in a "V" and "Y" formation resembling Chinese letters microscopically were identified as presumptive *Corynebacterium* species, and stored on nutrient agar slants at 4 °C until required for further processing. Species identity was determined using biochemical tests as previously

described by Reference [15] with further confirmation using MALDI-TOF (Brucker Daltonics, Germany)

B. Antimicrobial Susceptibility Test

The Kirby–Bauer disc diffusion method [16] with Mueller–Hinton agar was used to determine the sensitivity of the isolates to some commonly used antimicrobials. The antimicrobial agents tested include enrofloxacin (5 µg), gentamicin (10 µg), meropenem (10 µg), sulphamethoxazole/trimethoprim (25 µg), doxycycline (30 µg), nalidixic acid (30 µg), ampicillin (10 µg) and neomycin (10 µg). As a result of the nonavailability of standard disk diffusion method breakpoints from the CLSI for corynebacteria, the break point values of *Staphylococcus* species were used, while for ampicillin and meropenem breakpoint values of *Streptococcus* species were used [17], [18].

C. Coagulase Test

Tube coagulase test was performed to determine the ability of the *Corynebacterium* species to produce coagulase. The test was carried out as described in Cowan and Steel's Manual for the identification of medical bacteria [15].

D. Statistical Analysis

Descriptive statistics were used to report the results of isolation frequency and susceptibility testing. The results were presented in proportion and rates in tables.

III. RESULTS

Twenty-three strains of *Corynebacterium* species were isolated from 207 nasal swabs from slaughtered ruminants (cattle, sheep and goats) in Wurukum Abattoir, Makurdi (Tables I–III). Though the isolation rate of corynebacteria was far much higher in sheep than in cattle and goats (Table I), the association between *Corynebacterium* infection and animal species was not significant ($p < 0.05$). Sex prevalence of *Corynebacterium* infection shows that more females (12.1%) than males (8.6%) harbour the organism (Table II and Fig. 1). However, the association between *Corynebacterium* infection and sex of the animals was not significant ($p < 0.05$).

Six species were identified from the 23 *Corynebacterium* isolates, while 3 isolates were not identified at species level. The species of *Corynebacterium* identified are shown in Table III. *Corynebacterium xerosis* and *Corynebacterium amycolatum* were the most prevalent. *Corynebacterium xerosis* was identified in all the animal species, while *Corynebacterium amycolatum* was identified both in cattle and goats. Also, *Corynebacterium mycetoides* was isolated from sheep and goats, and *Corynebacterium stationis* was isolated from cattle and sheep. *Corynebacterium efficiens* and *Corynebacterium striatum* were respectively isolated only from cattle and goat.

The results of antibiotic susceptibility testing for the *Corynebacterium* species are presented in Table IV. The *Corynebacterium* isolates displayed high rates of resistance (31.6–100%) to all the antibiotics tested. *Corynebacterium xerosis* displayed high resistance rates, ranging from 42.9–100% to all the antibiotics tested except gentamicin with

resistance rate of 28.6%. All the strains of *Corynebacterium amycolatum* tested were susceptible to enrofloxacin and demonstrated a low resistance rate (25%) to neomycin. The strains of *Corynebacterium mycetoides* and *Corynebacterium stationis* were susceptible to gentamicin and neomycin and displayed a high level of resistance to all the other antibiotics tested. Also, the strains of the unidentified *Corynebacterium* species were susceptible to neomycin and demonstrated a high level of resistance to the rest of the tested antibiotics. The *Corynebacterium efficiens* strain was susceptible to nalidixic acid and resistant to the other antibiotics, while the strain of *Corynebacterium striatum* was resistant to all the antibiotics tested. Multidrug resistance to 3 or more antibacterial agents was observed for 15 (78.9%) of the isolates tested. A total of 14 resistance patterns were recorded for the 19 strains tested (Table V). Two strains of *Corynebacterium xerosis* were resistant to all 8 antibiotics tested, while the remaining 5 strains were resistant to at least 4 antibiotics. The only strain of *Corynebacterium efficiens* was resistant to 7 out of the 8 antibiotics tested, while that of *Corynebacterium stationis* was resistant to 6 antibiotics.

Eight (34.8%) out of the 23 *Corynebacterium* isolates were coagulase-positive. These coagulase-positive isolates comprise 6 strains of the *Corynebacterium xerosis* and the 2 strains of *Corynebacterium stationis*. The other strains were coagulase-negative.

TABLE I: PREVALENCE OF *CORYNEBACTERIUM* INFECTION IN SLAUGHTERED RUMINANTS IN MAKURDI

Animal species	Total no. of samples	No. (%) positive isolates
Cattle	91	12 (13.2)
Sheep	15	4 (26.7)
Goats	101	7 (6.9)
Total	207	23 (11.1)

% = Percent of total number of samples in each row.

TABLE II: SEX PREVALENCE OF *CORYNEBACTERIUM* INFECTION IN SLAUGHTERED RUMINANTS IN MAKURDI

Sex	Total no. of samples	No. (%) positive isolates
Female	149	18 (12.1)
Male	58	5 (8.6)
Total	207	23 (11.1)

% = Percent of total number of samples in each row.

TABLE IV: FREQUENCY OF ANTIMICROBIAL RESISTANCE OF *CORYNEBACTERIUM* SPECIES

Antibiotics	Number (%) ^a of resistant strains							Total n=19
	<i>C. xerosis</i> n=7	<i>C. amycolatum</i> n=4	<i>C. mycetoides</i> n=3	<i>C. stationis</i> n=1	<i>C. efficiens</i> n=1	<i>C. striatum</i> n=1	<i>C. species</i> n=2	
Gentamicin (10µg)	2 (28.6)	2 (50.0)	0	0	1 (100.0)	1 (100.0)	1 (50.0)	7 (36.8) ^b
Nalidixic acid (30 µg)	5 (71.4)	4 (100.0)	3 (100.0)	1 (100.0)	0	1 (100.0)	2 (100.0)	16 (84.2)
Neomycin (10 µg)	3 (42.9)	1 (25.0)	0	0	1 (100.0)	1 (100.0)	0	6 (31.6)
Sulphamethoxazole/ trimethoprim (25 µg)	6 (85.7)	2 (50.0)	2 (66.7)	1 (100.0)	1 (100.0)	1 (100.0)	1 (50.0)	14 (73.7)
Doxycycline (30 µg)	6 (85.7)	2 (50.0)	2 (66.7)	1 (100.0)	1 (100.0)	1 (100.0)	2 (100.0)	15 (78.9)
Enrofloxacin (5 µg)	5 (71.4)	0	1 (100.0) ^c	1 (100.0)	1 (100.0)	1 (100.0)	NA	9 (60.0) ^c
Meropenem (10 µg)	7 (100.0)	4 (100.0)	NA	1 (100.0)	1 (100.0)	NA	NA	13 (100.0) ^d
Ampicillin (10 µg)	7 (100.0)	4 (100.0)	NA	1 (100.0)	1 (100.0)	NA	NA	13 (100.0) ^d

^a(%) of total number of each species; ^b(%) of total number of isolates (19) tested; ^c = 15 isolates tested for enrofloxacin; ^d = 13 isolates tested meropenem and ampicillin; ^e = 1 strain of *C. mycetoides* tested for enrofloxacin; NA = not available.

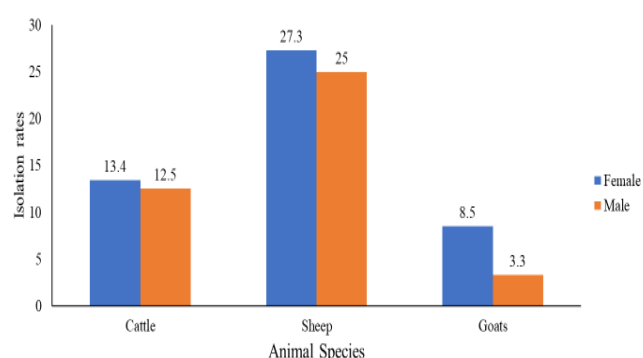


Fig. 1. Sex percentage occurrence of *Corynebacterium* in different ruminant species.

TABLE III: FREQUENCIES OF *CORYNEBACTERIUM* SPECIES ISOLATED FROM NASAL SWABS FROM SLAUGHTERED RUMINANTS

Identified Species	Frequency (%) ^a of species			Total (%) ^b
	Cattle	Sheep	Goats	
<i>Corynebacterium xerosis</i>	6 (75.0)	1 (12.5)	1 (12.5)	8 (34.8)
<i>Corynebacterium amycolatum</i>	3 (60.0)	0 (0.0)	2 (40.0)	5 (21.7)
<i>Corynebacterium mycetoides</i>	0 (0.0)	1 (33.3)	2 (66.7)	3 (13.0)
<i>Corynebacterium stationis</i>	1 (50.0)	1 (50.0)	0 (0.0)	2 (8.7)
<i>Corynebacterium efficiens</i>	1 (100.0)	0 (0.0)	0 (0.0)	1 (4.3)
<i>Corynebacterium striatum</i>	0 (0.0)	0 (0.0)	1 (100.0)	1 (4.3)
<i>Corynebacterium species</i>	1 (33.3)	1 (33.3)	1 (33.3)	3 (13.0)
Total	12 (52.2)	4 (17.4)	7 (30.4)	23 (100)

^a(%) of total number of isolates in each row; ^b(%) of total number of isolates (23).

TABLE V: ANTIBIOTIC RESISTANCE PATTERNS OF THE ISOLATES

S/No.	Pattern	Frequency (%)
1	NA+SXT	1 (5.3)
2	NA+DO	3 (15.8)
3	NA+MEM+AMP	1 (5.3)
4	CN+NA+SXT+DO	1 (5.3)
5	NA+N+SXT+DO	1 (5.3)
6	NA+SXT+DO+ENR	1 (5.3)
7	NA+SXT+MEM+AMP	1 (5.3)
8	CN+NA+N+SXT+DO	2 (10.5)
9	CN+NA+SXT+MEM+AMP	1 (5.3)
10	NA+DO+ENR+MEM+AMP	1 (5.3)
11	SXT+DO+ENR+MEM+AMP	2 (10.5)
12	NA+SXT+DO+ENR+MEM+AMP	1 (5.3)
13	CN+N+SXT+DO+ENR+MEM+AMP	1 (5.3)
14	CN+NA+N+SXT+DO+ENR+MEM+AMP	2 (10.5)

(%) = Percent of total number of isolates (19) tested

IV. DISCUSSION

In different parts of the world, opportunistic infections caused by non-diphtheritic corynebacteria, especially in immunocompromised patients, is on the rise; and these zoonotic bacteria are also associated with important animal diseases. The present study confirmed the presence of potentially zoonotic species of non-diphtheritic corynebacteria in slaughtered cattle, goats, and sheep in Makurdi, Benue State. Besides, *Corynebacterium efficiens*, which is a non-clinical glutamic-acid-producing species from soil and vegetables [19], other species isolated in this study have been associated with one disease or the other. In human, *Corynebacterium amycolatum* and *Corynebacterium striatum* which were identified in this study, have been implicated in bacteraemia, endocarditis and in acute/chronic complicated skin and soft tissue infections [2], [10], ear infection [8] and corneal ulcer [9]. Also, *Corynebacterium xerosis* isolated in this present study is among the most frequently reported coryneform bacteria causing infections in human [6], [20].

The isolates in this study demonstrated high level of antimicrobial resistance to some commonly used antimicrobials. This finding agrees with the reports of Reference [14] which suggests non-diphtheritic corynebacteria are becoming an emerging group of multidrug resistant bacteria. Similarly, our data on multidrug-resistant phenotypes agree with the study of Reference [2] in Romania where multidrug resistance phenotypes were displayed by *Corynebacterium* species. The antibiotics tested belong to different classes that are commonly used treatment options for diseases caused by *Corynebacterium* species. The high level of antibiotic resistance and unusual multidrug resistance profiles observed in our study could be due to misuse of antibiotics, such as frequent antibiotic therapy in livestock without recourse to proper veterinary examination and treatment. The findings in the present study also supports the assertion of Reference [21] that it is often difficult to predict antimicrobial resistance for strains of *Corynebacterium* species, thus the use of antimicrobial drugs for treatment of diseases caused by non-diphtheritic corynebacteria should be based on the results of sensitivity tests.

Reports have shown that some of the non-diphtheritic corynebacteria are a serious threat to a safe food supply and thus can easily be transmitted to humans when exposed to contaminated meat products. Therefore, the presence of these potentially zoonotic and multidrug resistant corynebacteria pose a serious threat to veterinary and public health in the study area. Abattoir workers are prone to infections with these bacteria during handling and processing of meat products; while abattoir units, especially the slaughter and storage units, are invariably contaminated. Abattoir workers and environment in turn become a source of contamination and infection to both human and animals. *Corynebacterium* species are known to be Psychrophilic and have been isolated from refrigerated food, bacon and retail mutton [22]. Thus, standard hygiene practices should be adopted to reduce or eliminate contamination of the abattoir environment and meat products, and infection of abattoir workers.

In conclusion, the study has provided preliminary evidence for the presence and circulation of multidrug resistant and potentially zoonotic *Corynebacterium* species among animals in Makurdi, Nigeria and thus the role of slaughtered cattle as reservoirs and source for dissemination should not be overlooked. We recommend the practice of high standard of hygiene in abattoirs be emphasised and the need for research on the pathogenicity and resistance mechanisms of these non-diphtheritic corynebacteria in the study area.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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