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Multidrug Resistant *Rhodococcus equi* in Donkeys (*Equus africanus asinus*) in Ganawari North Central Nigeria

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SUMMARY

Rhodococcus equi is an important cause of respiratory infections in equidae. The organism causes pneumonia in foal and could be isolated from diverse sources, including the nasal cavity which plays a critical role in the ecology of *Rhodococcus equi*. The demographic characteristics of the donkeys studied showed that 90% of the donkeys slaughtered during the period of sample collection were adult, while 50 and 50% were both male and female donkeys respectively. Isolates were identified as *Rhodococcus equi* based on phenotypic and biochemical characteristics observed. The susceptibility of *Rhodococcus equi* to antibiotics was determined by disk diffusion method using different disc concentrations of antibiotics as follows; erythromycin (15µg), streptomycin (25µg), kanamycin (30µg), cephalotin (30µg), methicillin (30µg), nalidixic acid (30µg), penicillin (25 units), ceftiofur (30µg). The antibiotic susceptibility test results obtained showed that the isolates were 100% resistant to cephalotin and penicillin, while 90%, 50% and 16.6% of the isolates were resistant to methicillin, erythromycin, streptomycin and nalidixic acid respectively. Only 16.6% of the isolates showed intermediate resistance to nalidixic acid. Interestingly, 100% of the isolates were sensitive to kanamycin and ceftiofur while 90%, 66.6%, and 50% were sensitive to streptomycin, nalidixic acid, and erythromycin. There is palpable public health risk that may arise from consumption of donkey meat contaminated with multidrug resistant *Rhodococcus equi*. Therefore, adequate food safety procedures should be enforced to protect human infection.

Key words: *Rhodococcus equi*, Donkey, *Caballus equi*, Antimicrobial Resistance, Multidrug resistance, North central Nigeria.

INTRODUCTION

Rhodococcus equi is a Gram-positive bacterium widespread in the environment of grazing farms. It is common in the faeces of wildlife and farm animals including swine, cattle, horses and others (Takai *et al*., 2002; Takai *et al*., 2003; Takai and Tsubaki,1985; Soedarmanto *et al*., 1988; Lara *et al*., 2015). The organism is widespread in the faeces of herbivores, especially horses, and their environment (Woolcock *et al*., 1980; Prescott *et al*., 1984). Inhalation and ingestion are considered routes of infection (Martens *et al*., 1982; Johnson *et al*., 1983). It is responsible for foal rhodococcosis – highly fatal pyogranulomatous bronchopneumonia that affects foals of age six months and below but rare in adult horses (Giguère, *et al*, 2011; Morresey and Waldridge, 2010).

Even though *Rhodococcus equi* is a known bacterial cause of pneumonia in equines, it is less considered as bacterial cause of pneumonia in donkeys. This is supported by the dearth of literature on rhodococcosis in donkeys. Recently, *Rhodococcus equi* infection in other species than horse has sparked considerable interest due to its frequent isolation from the lymph nodes and other tissues of apparently healthy animals intended for human consumption (Witkowski *et al*., 2016). This survey was therefore conducted to isolate, characterize and antimicrobial susceptibility of *Rhodococcus equi* in donkeys mostly slaughtered for food in Ganawuri, Plateau State, North Central Nigeria. The results of this study would add to the body knowledge on *Rhodococcus equi* as causative agent of bacterial pneumonia in equids with reference to donkeys in Nigeria.

MATERIALS AND METHODS

Study area

Samples were collected from Ganawuri in Ganawuri District, Riyom LGA, Plateau State. It is a class P populated place located on the following coordinates 9° 40’ 60” N and 8° 42’ 0” E. Ganawuri is located at an elevation of 1.265 meters above sea level and its population was total to 71,027. Its coordinates are 9° 40’ 60” N and 8° 42’ 0” E in DMS (Degree minute second) or 9.683°N and 8.700°E (in decimal degree). Its UTM position is MR67 and its joint operation graphic reference is NC32-10 (www.getamap.net accessed 18/3/2021).

Ethical approval

All samples used in this study was collected from slaughtered animals, thus ethical approval was not necessary since the process of sample collection was not invasive.

Sample collection

Each animal was identified individually, and its demographic information was recorded. The external part of the nose was disinfected with 70% alcohol, a sterile swab stick (Evepon sterile swab stick®, Nigeria) was moistened in normal saline and inserted into each nostril and rotated against the wall of the nasal cavity (Carter, 1984). The swab sticks were placed in a sterile sample bottle containing normal saline and kept in ice box and transported to the Pasteurella Research Laboratory, National Veterinary Research Institute, Vom for analysis.

Isolation, and identification of *Rhodococcus equi*

One hundred (n=100) donkeys were sampled after slaughter. The samples collected were aseptically inoculated onto blood agar, streaked and incubated at 37^{o}C for 24 hours. From the culture, positive plates colonies suggestive of *Rhodococcus equi* with the following colonial appearance; grey, small, or large, mucoid, non-

hemolytic were subjected to Gram's staining to study reaction and cellular morphology. Biochemical tests such as sugar fermentation, oxidase, catalase and urease tests were performed as described (Takai and Tsubaki, 1985). The organisms were identified based on phenotypic characteristics, colony morphology and biochemical tests as described by Takai and Tsubaki (1985).

In vitro antimicrobial susceptibility testing

Disk diffusion assay was performed on each isolate and this was carried out as described by Bauer et al. (1959). Results were interpreted in accordance with the guidelines established by the Clinical and Laboratory Standard Institute (CLSI, 2017).

RESULTS

The demographic characteristics showed that 90% of the donkeys slaughtered during the period of sample collection were adult, and 50% were both male and female donkeys respectively (Table 1).

Table 1: Zoographic information of Rhodococcus Equi positive Donkeys (*Equus africanus asinus*).

Sample ID No.	Sex	Age	Sample type
GNR/2020/099	Female	Adult	Nasal swab
GNR/2020/045	Male	Young	Nasal swab
GNR/2020/045	Male	Adult	Nasal swab
GNR/2020/052	Female	Adult	Nasal swab
GNR/2020/082	Female	Adult	Nasal swab
GNR/2020/096	Male	Adult	Nasal swab

Isolates were identified as *Rhodococcus equi* based on phenotypic and biochemical characteristics observed (Table 2).

The susceptibility of *Rhodococcus equi* to various antibiotics was determined by disk diffusion method. The results are as shown (Table 3 and 4).

The demographic characteristics showed 90% of the donkeys slaughtered during the period of sample collection were adult, and 50% were both male and female donkeys respectively. The only young male donkey in the slaughter figure during the sample collection was positive for *Rhodococcus equi*. While equal number of male and female were observed among the carriers of *Rhodococcus equi*.

In this survey, *Rhodococcus equi* was isolated and identified based on its phenotypic, biochemical and morphological characteristics. The isolates grew on blood agar at incubation temperature of 37°C for 24 hours presenting grey or creamy colours, mucoid, large to small colonies that were non haemolytic on blood agar. However, the colonies were positive for catalase, urease and negative for oxidase perhaps they failed to ferment sugars even after prolonged incubation. Microscopically all the isolates were gram positive cocci. These characteristics were consistent with *Rhodococcus equi* as earlier described (Takai and Tsubaki, 1985; Rzewuska *et al.*, Witskowski *et al*., 2016).

The results showed the isolates were 100% resistant to Cephalotin, Penicillin, and 90% Methicillin, 50% Erythromycin, 16.6% Streptomycin and Nalidixic acid respectively. Only 16.6% of the isolates showed intermediate resistance to Nalidixic acid.

Table 2: Microscopic and Biochemical Characteristics of Rhodococcus equi isolated from Donkeys (Equus africanus asinus) slaughtered in Ganawuri

Sample ID.	Microscopic appearance	Catalase	Oxidase	Urease	Glu	Suc	Lac	Mal	Man	Xyl	Rha
GWR/2020/099C	Gram positive cocci	+	-	+	-	-	-	-	-	-	-
GWR/2020/045C	Gram positive cocci	+	-	+	-	-	-	-	-	-	-
GWR/2020/045G	Gram positive cocci	+	-	+	-	-	-	-	-	-	-
GWR/2020/052C	Gram positive cocci	+	-	+	-	-	-	-	-	-	-
GWR/2020/082C	Gram positive cocci	+	-	+	-	-	-	-	-	-	-
GWR/2020/096G	Gram positive cocci	+	-	+	-	-	-	-	-	-	-

+ = positive, - = negative, Glu = Glucose, Suc = Sucrose, Lac = Lactose, Mal = Maltose, Man = Mannose, Xyl = Xylose, Rha = Rhamnose

Table 3: Antibiogram (mean ± SD) of Rhodococcus equi isolated from Donkeys (Equus africanus asinus) in Ganawuri

Mean (± SD) Antibiotic Susceptibility (mm)								
Isolate ID No	Erythromycin	Streptomycin	Kanamycin	Cephalotin	Methicillin	Nalidixic acid	Penicillin	Ceftiofur
GWR/2020/099C	30.0 ± 0	21.0 ± 0.81	21.0 ± 0.81	16.5 ± 0.04	22.0 ± 0	28.0 ± 0	21.0 ± 0.81	30.0 ± 1.63
GWR/2020/045C	7.5 ± 0.04	22.0 ± 0.81	23.5 ± 1.22	6.0 ± 0	6.0 ± 0	9.0 ± 0.81	6.0 ± 0	32.0 ± 0.81
GWR/2020/045G	12.0 ± 0	24.0 ± 0	26.0 ± 1.63	7.5 ± 1.22	6.0 ± 0	14.5 ± 0.40	6.5 ± 0.40	37.0 ± 0.81
GWR/2020/052C	6.0 ± 0	14.5 ± 0.4	19.0 ± 0.81	6.0 ± 0	6.0 ± 0	28.5 ± 0.40	6.0 ± 0	27.0 ± 0
GWR/2020/082C	34.0 ± 0	30.0 ± 0	30.0 ± 1.63	14.0 ± 0	15.0 ± 8.98	25.5 ± 0.81	34.0 ± 0	31.0 ± 0.81
GWR/2020/096G	28.0 ± 1.63	10.0 ± 0	25.0 ± 0.81	6.0 ± 0	7.0 ± 0.81	42.0 ± 1.63	10.0 ± 0	26.0 ± 3.26

Concentration of antibiotic disc: Erythromycin (15µg), Streptomycin (25µg), Kanamycin (30µg), Cephalotin (30µg), Methicillin (30µg), Nalidixic acid (30µg), Penicillin (25U), Ceftiofur (30µg)

Table 4: Susceptibility profile of Rhodococcus equi isolated from Donkeys (Equus africanus asinus) in Ganawuri

Susceptibility profile Rhodococcus equi								
Isolate ID No	Erythromycin	Streptomycin	Kanamycin	Cephalotin	Methicillin	Nalidixic acid	Penicillin	Ceftiofur
GWR/2020/099C	S	S	S	R	S	S	R	S
GWR/2020/045C	R	S	S	R	R	R	R	S
GWR/2020/045G	R	S	S	R	R	I	R	S
GWR/2020/052C	R	S	S	R	R	S	R	S
GWR/2020/082C	S	S	S	R	R	S	R	S
GWR/2020/096G	S	R	S	R	R	S	R	S

Sensitive (S), Intermediate (I), ID Resistance

DISCUSSION

Rhodococcus equi is a Gram-positive, aerobic, non-motile, non - sporulating and metabolically diverse bacterium (Ocampo-Sosa *et al*., 2007). It has been recognized as a pulmonary pathogen of horses. However, humans, equines and other animal species such as ruminants, pigs, cats and dogs are also affected by the organism (Vázquez-Boland *et al*, 2013). It is known for zoonotic infections in foals that are between 1 to 4 months of age (Takai, *et al*, 2002). It is ranked among the top most important pathogens in the horse industry especially because of its high prevalence and mortality rate (Giguère *et al*, 2011).

We did a short survey to isolate *Rhodococcus equi* in hundred (n=100) donkeys. Nasal swabs were collected from donkeys slaughtered for food at Ganawuri, Riyom local government area in Plateau State North Central Nigeria. Even though PCR is a rapid and sensitive method for the detection and identification of bacteria, we couldn't perform PCR to identify our isolates, bacterial culture has been considered to be the most valuable method for routine diagnosis of *Rhodococcus equi* pneumonia in foals (Anzai *et al*., 1997; Javed *et al*., 2017; Viverette *et al*., 2000).

Isolation of the organism in young donkey although not a foal was in congruence with earlier reports (Giguere *et al*., 2010; Mooresey *et al*., 2010). That only foal up to six months of age have a unique susceptibility to *Rhodococcus equi*. Similarly, the isolation of *Rhodococcus equi* in adult donkeys agrees with the reports of Witkowski *et al*. (2016). They have characterized *Rhodococcus equi* isolated from adult horses slaughtered for food in Poland. This indicated that *Rhodococcus equi* is both a pathogen of adult and young Equidae but occurs more frequently in young but rarely in adult equids. Foals are prone to infection being immunocompromised at younger ages.

The susceptibility of the isolates to various antibiotics was determined using disk diffusion method and interpreted according to the guidelines of CLSI (2018); weney et al. (1997) had reported resistance to cephalothin in a study of *Rhodococcus equi* pneumonia in 48 foals. Similarly, clinical isolates of *Rhodococcus equi* have frequently been found to be resistant to penicillin, oxacillin, tetracyclines, ampicillin, , trimethoprim/sulphadiazine and second- and third generation cephalosporins (Ellenberger and Genetzky, 1986; Nordmann and Ronco, 1992). In this study 90% of our isolates were resistant to methicillin. Javed *et al*. (2017) had reported 66.6% of their isolates were resistant to methicillin. However, MIR et al. (2015) has documented 50% of their isolates being resistant to penicillin G. Various studies have reported resistance to erythromycin which was a drug of choice for the treatment of rhodococcosis infection in horses and humans in combination with rifampicin (Erol *et al*., 2019). Giguère *et al*, (2010) had observed resistance to erythromycin (and azithromycin, clarithromycin, and rifampin) in 22 in foals infected with antimicrobial-resistant isolates of *Rhodococcus equi*. In the same vein Huber *et al*. (2019) had observed resistance to erythromycin in clinical isolates of *Rhodococcus equi* from foals in Central Kentucky studied from 1995 to 2017. Resistance to streptomycin and kanamycin was also observed by MIR *et al*. (2015) in their study.

Interestingly, 100% of the isolates were sensitive to kanamycin, ceftiofur, and 90% to streptomycin,

66.6% nalidixic acid, and 50% erythromycin (Table 3 and 4). Mir et al. (2015) has observed 100% and 58.3% sensitivity to erythromycin and kanamycin respectively, this corroborates our result in this study. That *Rhodococcus equi* were found to be sensitive to kanamycin and erythromycin. Our isolates showed 100% sensitivity to ceftiofur a third-generation cephalosporin has been shown to be active against *Rhodococcus equi* in vitro (Jacks et al. 2003).

In general, the results showed our isolates were multi-resistant and reports on *Rhodococcus equi* as multi-resistant pathogen has been trending not only as a pathogen causing pneumonia in foal or adult horses and donkeys, but as a zoonotic pathogen that resists antimicrobials in humans is worrisome. Extreme caution should be taken while dealing with this organism.

To our knowledge, there is no published literature reporting *Rhodococcus equi* in horses or donkeys in Nigeria, as such this represents the first report on the isolation of this bacterium in apparently healthy donkeys brought for slaughter in Ganawuri in Plateau State, North central Nigeria.

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