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Streptococcus suis in Pigs and Environs: A Cross-sectional Study

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Abstract

Streptococcus suis has been reported as the most causative agent of streptococcal infection of pigs and humans among the pig farmers in Nigeria, although the common reservoir of this infection is not really specified. This study was carried out to x-ray the cross-sectional study of *S. suis* among pigs and environs. A total of 430 samples were drawn from different sources (nasal swab, pork, pig droppings, pig feeds, bioaerosol), and screened for the presence of *Streptococcus suis* using standard microbiological techniques. The isolates obtained were characterized using their morphological and biochemical characteristics. The study revealed the presence of *Streptococcus suis* Q, *Streptococcus suis* R, *Streptococcus suis* S and *Streptococcus suis* Y, of which the isolates were significantly ($\alpha<0.05$) seen most in nasal swab samples whereas the isolates were seen least in pig feed samples. *Streptococcus suis* Q was most significantly ($\alpha<0.05$) seen among the samples whereas *S. suis* R was seen least. From the study, *S. suis* was seen in the studied samples and it was harbored most in nasal samples, and *S. suis* Q was seen most in the studied samples.

Keywords: *Streptococcus suis, Streptococcal infection, Pig infection, Pig farmers*

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Introduction

Streptococcus species are quantitative anaerobes, non-spore formers, Gram-positive rods and catalase-negative bacteria. *Streptococcus* species are common endemic pathogens in pigs raised in different environments majorly in developing countries (Feng *et al.*, 2004; Segura *et al.*, 2016). The organisms cause infectious diseases that are considered to be multifactorial; i.e the infection may remain subclinical or lead to clinical infection depending on several factors such as overcrowding, poor ventilation, climate conditions, poor hygiene status, high air pollution load and other Stresses (Ma *et al.*, 2008). Furthermore, host-specific factors, such as age, genetic background and disease conditions also affect the disease induction (Ma *et al.*, 2008). Studies have shown that weaning piglets are most susceptible to this infection since protective maternal antibodies decline (Ma *et al.*, 2008). *Streptococcus suis* (Lancefield group D) is a frequent early colonizer of the upper respiratory tract of pigs. It has been associated with septicemia, meningitis, polyserositis, endocarditis and pneumonia in young pig (Goltschelk *et al.*, 2010; Goyette-Desjardins *et al.*, 2014; Segura *et al.*, 2016). The organism is commensally in the respiratory part of the pigs, particularly in the tonsils and nasal clarities but transitions to other parts, confection with other pathogens and some predisposing factors trigger the organism to cause disease in the pigs (Feng *et al.*, 2004; Segura *et al.*, 2016). Suppurative arthritis associated with Lancefield group C (usually *Streptococcus equisimilis* has been reported, (Opriessnig *et al.*, 2011). Human causes of *Streptococcus* species, majorly *S. suis* are infrequent but severe, meningitis is the most common manifestation of *S. suis* infection in humans followed by septicemia and endocarditis. These diseases are often mistaken for other pathogens due to lack of awareness of the existence of this pathogen (Meng *et al.*, 2015, Bojarska *et al.*, 2016; Segura *et al.*, 2016). In most cases, *S. suis* capsular type Z is commonly responsible for the infection, and this can be acquired through close contact, with the pigs, infected pork and the products, contaminated drinking water and bioaerosols, and the organism gets access to humans through skin abrasions and cuts (Bojarska *et al.*, 2016). Diarrhea is the primary sign associated with *Enterococcus durans* infections, usually in neonate pigs (2-14 days old). Streptococcal lymphadenitis caused by *streptococcus porcinus* in growing pigs is associated with abscess formation on the jowl or in the cervical region. The infection is due to dissemination of the organisms from its initial location in the pharynx and tonsils, and this is usually acquired via. Faecal-oral-route, direct contact or contaminated drinking water (Segura *et al.*, 2004; Gohsclalk *et al.*, 2010).

In Nigeria, majorly in Ihiala Local Government Area in Anambra State, distribution of Streptococcus suis among the pigs and their associated samples were neglected, little or no published study had been done in order to ascertain the possible measures to curb the menacing effects of this organism among pigs and pig farmers. Therefore this need to conduct a cross-sectional study on the occurrences of the organism among pigs and the environs for better elimination or eradication measures

Materials and Methods

Study Area: The study was carried out in Ihiala LGA Anambra state. Ihiala is situated at Latitude 5.85°N and Longitude 6.86°E, with an elevation of 144

m above sea level. It is located 48 Km North of Owerri and 40Km south of Onitsha. It covers an area of 304 SqKm and is bounded by Ogbaru (in Ogbaru LGA, Anambra state) on the west, Ozubulu (in Ekwusigo LGA, Anambra State), Ukpor and Osumenyi (in Nnewi south LGA Anambra state) in the North and in the South by Egbuoma, Ohakpu, Ozara and Oguta in Egbema/Oguta LGA of Imo state. Ihiala has a tropical climate (rainy and dry seasons) with double maximal rainfall. The rainy season is between April and October, and the dry season is between November and March. The annual rainfall ranges from 1800 mm to 2000 mm. The major anthropological activities are farming/agriculture and trading, of which Pig farming is one of the major farming practices. In this study, samples were collected from the major towns in Ihiala LGA, which included Amorka, Azia, Lilu, Okija, Mbosi, Isseke, Orsumoghu, Ubuluisiuzor and Uli.

Sample Collection: A total of 430 samples which comprises 120 nasal swab samples 60 pork samples, 120 Pig droppings, 90 pig feeds samples, and 40 bioaerosol samples were used for this study.

Pork Samples: Ready to eat samples was aseptically collected using a sterile stainless spoon (Hamada) into a sterile aluminum foil from different selling locations in different towns in Ihiala LGA (Wang *et al.*, 2012)

Nasal Swab Samples: The pig's nasal septum and the environs were cleaned with cotton wool soaked in 70% ethanol. The swab stick soaked in normal saline was aseptically inserted into the nasal cavity of the pig. The swab was turned both clockwise and anticlockwise direction for effective collection. This was carefully withdrawn and inserted into the pack containing the sterile nutrient broth in order to enrich the organisms in the samples. This was repeated for every pig (Espinosa-gongura *et al.*, 2016)

Pig Dropping Samples: These were aseptically collected 2.0 m away from each dropping in a particular pigsty using a sterile stainless spoon (Hamada) and polythene bag. Then the collected samples in a particular pigsty were carefully and aseptically mixed together as a representative sample for analysis. This was repeated at different locations (Marti *et al.*, 2009).

Pig Feed Samples: These were randomly collected from the top, middle and bottom of the bag at a particular site using a sterile stainless spoon (Hamada) and polythene bag. The collected samples were carefully and aseptically pooled together as a representative sample for analysis. This was repeated at different sampling locations (Kookier *et al.*, 2012, Wang *et al*, 2012)

Bioaerosol Samples: This was carried out using the sedimentation method. Sterile disposable petri dishes (90 mm X 15 mm) containing 20 mL of sterile solidified blood agar medium were carefully and aseptically exposed at different elevations (floor, tables and cabinets) and positions in the pigsty for 30 minutes and the plates were covered for microbial enumeration. The same procedure was repeated for every sampling location (Tshokey *et al.*, 2016; Haas *et al.*, 2017).

Transportation of the Samples: Here, a cooler containing an ice block wrapped in a sterile polythene bag was used for the sample transportation. The temperature of the cooler was checked and adjusted to 28ºC-30ºC by reducing the quantity of the ice inside the cooler in order to reduce or prevent microbial shock. The samples were carefully and aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was then covered and the drain plug was securely taped with packing tape to prevent accidental opening of the cooler. The cooler was then safely carried to the Laboratory for analysis within 2 h of sample collection. The same procedure was repeated for other collection times (Wolking and Davis, 2013).

Sample Preparation: This was carried out using the routine laboratory technique. The pork sample was ground using a sterile blender (LXB 242). The 1.0g each of the ground sample pig droppings and pig feeds were aseptically weighed into a 10ml test tube (Pyrex) each respectively, about 3ml of normal saline was aseptically added into each test tube and these were shaken thoroughly and then made up to 10.0 mL using the normal saline for each test tube.

Isolation of test organisms from the samples: The prepared samples (pork, pig droppings and pig feeds) were aseptically grown in blood agar (BIOTECH) which was prepared according to the manufacturer's instructions and the procedures described in Cheesbrough (2010) and Frank and Robert (2015). The nasal samples were aseptically streaked in sterile poured blood agar plates (90 mm×15 mm) as described by Frank and Robert (2015). The same blood agar was used for the collection of air microbes using sedimentation techniques as described by Tshokey et al. (2016) and Hass et al. (2017). The cultured plates were carefully placed inside the bacteriological incubator (ST×B128) in an inverted position and incubated at 35±2ºC for 24 h.

Purification of the Isolates: The plates that showed discrete colonies were selected after 24 h and aseptically streaked each colony on a sterile poured plate (90mm×15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions and incubated at 35±2ºC for 24 h as described in Cheesbrough (2010) and Goldman and Green (2009).

Characterization of the Pure Isolates: This was carried out using the morphological and biochemical of the isolates as described by Iheukwumere *et al.* (2018).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described in Goldman and Green (2009). The gram staining technique which revealed the gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Cheesbrough (2010), Goldman and Green (2009) and Frank and Robert (2015). The presence or absence of capsule was also carried out as described by Goldman and Green (2009). The presence or absence of flagellum was determined by carrying out motility test as described by Cheesbrough (2010).

Biochemical characteristics of the isolates: The ability of the isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55% NaCl and to utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, sacharose, glucose trehalose, lactose, starch, inulin, salicin, hiparate) and also the kaemolytic activity of the isolates were carried out using the methods described by Cheesbrough (2010), Goldman and Green (2009) and Frank and Robert (2015).

Determination of prevalence of the isolates in the studied samples

The occurrences of different strains of *Streptococcus* species that were encountered in nasal swab, pork, pig feeds, pig droppings and bioaerosol samples were countered and recorded according to Iheukwumere *et al.* (2018)

Statistical Analysis

The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. Chi-square (x^2) was used to determine the significance of the sample sources. The significance of the prevalence of the isolates in the studied samples was determined at 95% using one-way analysis of variance (ANOVA). A pairwise comparison was carried out using the student "t" test..

Results

Sample Sources and Occurrences of the Isolates

A total of 430 samples were collected from five different sources of which the nasal swab Pig dropping samples were collected more (Table 1). The study revealed that the implicated isolates were detected most from bio-aerosol samples whereas the lowest detection was seen in Pig droppings. Also a total of 103 samples were positive to the implicated isolates whereas 327 samples were negative. The study further showed that there was association between the sources of the samples collection and the occurrences of the isolates as the data obtained were statistically significant ($\alpha < 0.05$).

Morphological Characteristics of the Isolates

The present study revealed that isolates Q, R, S and Y exhibited similar growth elevation. They were gram-positive, cocci, and non-motive. Isolates Q and S showed a grey-white appearance on blood Agar with smooth edge, shiny and mucoid and capsulated. Isolates S and Y showed a whitish appearance on Blood Agar, with entire edge, glisten and non-capsulated. The isolates were arranged in diplo and short chains except isolate S which was only arranged in short chains.

Biochemical Characteristics of the Isolates

The study showed that the isolates exhibited alpha (α) haemolysis on blood agar. They were negative to catalase, indole, voges prokauer (VP) and oxidase. They were not able to utilise hiparate, sorbitol, ribose neither did they grow on 6.5% NaCl. They utilized esculin, lactose, glucose, trehalose, saccharose and starch. Isolate Q, R and Y utilized inulin whereas isolate S showed slight utilization. Isolates Q and Y exhibited similar characteristics in the utilization

of salicin (slight) whereas the characteristics of isolate S was not able to utilize salicin. Isolates S and Y utilized arabinose whereas isolates Q and R showed slight utilization to arabinose.

Prevalence of the Bacterial Isolates in the Studied Samples

The study showed that a total of 103 isolates were seen in the studied samples, of which nasal swab harbored most of the isolates whereas the least occurrence was seen in Pig feeds. The study further showed that there was an association between the sources of the sample collection and the occurrences of the isolates as the data obtained from the data were statistically significant ($\alpha<0.05$). It was also observed that 4 strains of *Streptococcus suis* Q, R, S, and Y were implicated in the study and isolate Q was most significantly ($\alpha<0.05$) detected in the studied samples. Isolate R was not detected in pork, pig droppings and pig feeds. Isolate Y was detected in pig feeds, Isolate Q and Y were detected in all the studied samples and nasal swab samples harbored all the identified bacterial isolates (Table 4).

Table 1: Sample sources and occurrences of the isolates

Source	Total Sample	Positive (%)	Negative (%)
Nasal Swab	120	38(31.67)	82(68.33)
Pork	60	17(28.33)	43(71.67)
Pig droppings	120	13(10.83)	107(89.17)
Pig feeds	90	11(12.22)	79(87.78)
Bio-aerosol	40	27(60.00)	16(40.00)
Total	430	103(23.95)	327(76.05)

$X^2(31.03) > CV\ (9.49);\ \alpha < 0.05$

Table 2: Morphological characteristics of the isolates

Parameter	Isolate Q	Isolate R	Isolate S	Isolate Y
Colour of the isolate on BA	Grey white	Whitish	Grey white	Whitish
Size (mm)	1.80	1.50	1.70	1.60
Elevation	Convex	Convex	Convex	Convex
Edge	Smooth	Entire	Smooth	Entire
Appearance of the surface	Shiny and mucoid	Glisten	Shiny and mucoid	Glisten
Gram reaction	Positive	Positive	Positive	Positive
Cell Morphology	Coccus	Coccus	Coccus	Coccus
Cell Arrangement	Diplo and short chains	Diplo and short chains	Short chains	Diplo and short chains
Capsule	Positive	Negative	Positive	Negative
Motility	Negative	Negative	Negative	Negative

BA= Blood Agar

Table 3: Biochemical characteristics of the isolates

Parameter	Isolate Q	Isolate R	Isolate S	Isolate Y
Catalase	-	-	-	-
Indole	-	-	-	-
V.P	-	-	-	-
Oxidase	-	-	-	-
Growth on 6.5% NaCl	-	-	-	-
Esculine	+	+	+	+
Inulin	+	+	+	+
Lactose	+	+	+	+
Glucose	+	+	+	+
Trehalose	+	+	+	+
Hiparate	-	-	-	-
Salicin	+/-	+	-	+/-
Sacharose	+	+	+	+
Starch	+	+	+	+
Arabinose	+/-	+/-	+	+
Sorbitol	-	-	-	-
Ribose	-	-	-	-
Haemolysis	α	A	α	α

+= Positive; - = negative

Table 4: Prevalence of the bacterial isolates in the studied samples

Source	Isolate Q (%)	Isolate R (%)	Isolate S (%)	Isolate Y (%)	Total
Nasal Swab	19 (50.00)	3 (7.89)	7 (18.42)	9 (23.68)	38
Pork	10 (58.82)	0 (0.00)	1 (5.88)	6 (35.29)	17
Pig Droppings	8 (66.67)	0 (0.00)	1 (8.33)	3 (23.08)	12
Pig Feeds	7 (63.64)	0 (0.00)	0 (0.00)	4 (36.36)	11
Bioaerosol	14 (58.33)	1 (4.17)	3 (12.50)	6 (25.00)	24
Total	58 (56.31)	4 (3.88)	12 (11.65)	28 (11.65)	103 (100.00)

Discussion

The occurrences of different strains of *streptococcus suis*_in pork, pig droppings, pig feeds, nasal swab, from the pigs and the pig's bioaerosol supported the findings of many researchers (Papatsiros *et al*, 2011; Gottschalk *et al.*, 2013; Goyette-Desjardins *et al.*, 2014; Gustarrson and Rasmussen, 2014; Looft *et al.*, 2014; Takeuchi *et al.*, 2017; O'Dea *et al.*, 2018; Votsch *et al.*, 2018; Meng *et al.*, 2019; Segura *et al.*, 2020). According to Segura *et al*, 2020; *Streptococcus suis* is an early colonizer of the upper respiratory tract of piglets majorly during the birth canal. Takeuchi *et al*, 2017; reported the impact of a food safety campaign on *Streptococcus suis* infection in humans. The highest occurrences of *Streptococcus suis* in bioaerosol agrees with the report of Burrows *et al.* (2009); who highlighted that *Streptococcus suis* is biogenic aerosol particle that are ubiquitous in the atmosphere, and due to its size, it has a long atmospheric residence and can be transmitted by wind over long distances. The morphological, biochemical and molecular characteristics of *Streptococcus suis* isolated from the studied samples corroborated with the findings of many researchers (Groves *et al.*, 2015; Hatrongjit *et al.*, 2016; Okura *et al.*, 2016; O'Dea *et al.*, 2018;). Generally, the organisms were gram-positive, cocci, non-motile, gray-white/whitish on blood agar, α-haemolytic, catalase-negative but had variation in their ability to utilize sugars which indicates varying strains. Hence, the occurrences of *Streptococcus suis* strain Q, *Streptococcus suis* strain R, *Streptococcus suis* strain S and *Streptococcus suis* strain Y supported the findings Groves *et al.* (2015); Hatrongjit *et al.* (2016); Okura *et al.* (2016); O'Dea *et al.* (2018) who isolated different strains of *Streptococcus suis* and characterized them in their respective studies.

The occurrences of isolate Q, R, S and Y in the studied samples agree with the findings of many researchers (Goyette-Desjardins, 2014; O'Dea *et al.*, 2018; Kerdson *et al.*, 2020; Segura *et al.*, 2020). The highest occurrence of isolate Q in the studied sample could be attributed to diversity and survival rates of the isolates. The absence of Isolate R in the pork, pig droppings and pig feeds could be attributed to low diversity and poor survival rate of the isolate in the samples. Mural *et al*, (2017) and Preetha and Narayunan (2020) reputed that intrinsic factors play a vital role in determining the existence and survival of microbes in any sample.

Conclusion

The present study has shown that *Streptococcus suis* strain Q, *Streptococcus suis* strain R and *Streptococcus suis* strain S and *Streptococcus suis* strain Y were the implicated isolates in the nasal, pork, pig droppings, pig feeds and bioaerosol samples, of which isolate Q was predominant, and nasal samples were mostly implicated.

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