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EPIDEMIOLOGY OF MULTI-ANTIBIOTIC RESISTANT STREPTOCOCCUS SUIS IN PIG POPULATIONS AND FARM SURROUNDINGS

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ABSTRACT

Streptococcus suis is a significant zoonotic pathogen commonly found in pigs and their environments, and it is increasingly recognized for its role in human infections and antibiotic resistance. Notably, up to 80% of antibiotic resistance genes in S. suis are plasmid-encoded, raising concerns about its contribution to the spread of resistance. This study aimed to evaluate the antibiotic resistance patterns of different S. suis strains isolated from pigs and their surrounding environments. Samples were collected from various anatomical sites of pigs and nearby areas and analyzed using standard microbiological methods for the identification of S. suis. Antibiotic susceptibility testing was conducted using the disk diffusion method. The isolates were identified as S. suis Q, R, S, and Y. Resistance rates among these strains were 24.14% (S. suis Q), 100.00% (S. suis R), 41.67% (S. suis S), and 39.23% (S. suis Y). Furthermore, 64.29% of Q, 100.00% of R, and 63.64% of S isolates exhibited multiple antibiotic resistance (MAR), with 61.76% of the resistant strains having MAR indices greater than 0.2. Among the isolates, S. suis Q was the most prevalent, particularly in nasal samples. These findings highlight the presence of diverse, multiple antibiotic-resistant S. suis strains in pig populations and their environments, underscoring the potential public health risks and the need for vigilant monitoring and control strategies.

KEYWORDS

Streptococcus suis, Antibiotic Resistance, Zoonosis, MAR Index, Swine Pathogen

Introduction

Meanwhile, the knowledge of antibiotic susceptibility pattern of bacterial pathogens is required for overcoming the antimicrobial resistance pattern, and Studies have shown that pigs suffering from other bacterial and/ or viral it involves geographical variation, the information of antibiotic susceptibility infections of the upper respiratory tract are more susceptible to Streptococcal of *Streptococcus* species, majorly *S. suis* strains in Nigeria especially in infection, particularly *S. suis* infection (Chabot-Roy *et al.,* 2006; Lin *et al.,* Anambra State is limited if there is

actually any documented report at all. 2015; Meng *et al.*, 2015). In humans, especially advanced age and pre-existing.

Several studies from other parts of the world have revealed that antibiotic medical conditions that suppress the immune system, *streptococcal* infections resistance associated with *Streptococcus species* is plasmid-mediated are usually symptomatic (Ma *et al.*, 2008; Gnenier *et al.*, 2009). Also, the

(Iheukwumere *et al*., 2020). Therefore, the need for reversion using natural interactions of *S. suis* with the mucosal immune system and evasion of innate products from the botanical origin, which is cost-effective, ecofriendly and immune defense mechanisms are concila for induction of disease. *S. suis* has easily available in the environment to restore the hope of pig farmers toward several immune evasion strategies such as expression of polysacchanide achieving their goals, and to minimize antibiotic-mediated streptococcal capsule to prevent phagocytosis-dependent killing mechanisms infection in humans, will be ultimate success to be attained and calls for a (Yonykiettraknl *et al.*, 2019) or biofilm formation which may protect *S. suis* scientific approach.. from antimicrobial substances (Bojarska *et al.*, 2016). The prophylaxis and treatment of emerging zoonotic *streptococcal* infections in agriculture and health care setting mainly rely on antibiotics, and these antibiotics.

Materials and Methods

Meta Lactam (Penicillins, cephalosporins) and fluoroguinones

Study Area: The study was carried out in Ihiala LGA Anambra state. Ihiala (Ciprofloxacin) are the same in both pigs and humans (Yongkiettrakul *et al.*, is situated at Latitude 5.85^{o}N and Longitude 6.86^{o}E, with an elevation of 144 2019). However, the continuing use of these antibiotics contributes to the m above sea level. It is located 48 Km North of Owerri and 40Km South of emergence and widespread of antibiotic-resistant strains. The increase in these Onitsha. It covers an area of 304 SqKm and is bounded by Ogbaru (in Ogbaru resistant strains isolated from pigs and humans have been reported from many LGA, Anambra state) on the West, Ozubulu (in Ekwusigo LGA, Anambra countries in America, Asia and Europe (Yongkiehrakul *et al.*, 2019). Notably, State), Ukpor and Osumenyi (in Nnewi south LGA Anambra state) in the resistant *S. suis* has been identified as a reservoir for antibiotic resistance genes North and in the South by Egbuoma, Ohakpu, Ozara and Oguta in which can be transferred horizontally to streptococcal human pathogens such Egbema/Oguta LGA of Imo state. Ihiala has a tropical climate (rainy and dry as *S. pyogenes. S. pneumonia* and *S. agalactiae* (Yongkiettrakul *et al.*, 2019). 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.

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±2^{o}C 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±2º C for 24 h as described in Cheesbrough (2010) and Goldman and Green

(2009). The purified isolates were characterized based on the morphological and biochemical of the isolates as described by Iheukwumere *et al.* (2018).

Preparation of test isolate: The test isolates were prepared using the method described by Iheukwumere *et al.* (2017). The isolates were aseptically subcultured into a broth culture and incubated at 35±2º C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to turbidity that matched 0.5 Macfarland standard that was prepared by mixing 0.6ml of 1% $BaCl_2$ $2H_2O$ and 99.4 mL of 1% Conc H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 Macfarland standards at 640 nm using a UV/visible spectrophotometer.

In *vitro* activity of conventional antibiotics against the isolates using disc diffusion method

The susceptibility of the isolates to the conventional antibiotics was carried out by the disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37˚C for 24 h. Meter rule was used underside of the plates to measure the diameter zones of inhibition in millimeter.

Data 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, susceptibility patterns and degree of resistance of the isolates at 95% confidence level. The significance of the prevalence of the isolates in the studied samples was determined at 95% using a one-way analysis of variance (ANOVA). A pairwise comparison was carried out using the student "t" test.

Results

Conventional Antibiotic Susceptibility Patterns of the Isolates

The study showed that 66.02% of the total isolates were susceptible to conventional antibiotics whereas 33.68% were resistant. The study also revealed that 100% of isolate R were resistant followed by isolate Y, S and Q showed resistance to the conventional antibiotic. The data obtained from this study showed a statistical significance difference ($P<0.05$) between the strain of bacterial isolates and susceptibility patterns to conventional antibiotics as shown in Table 1.

Degree of Resistance Exhibited By the Isolates

The present study showed that 32.35% of the resistance bacterial isolates (SAR) whereas 67.65% exhibited multiple antibiotic resistance (MAR). It was observed that 100% of isolate R exhibited MAR (Table 2). The study further showed that there was an association between the strains of the organism and the degree of resistance exhibited against conventional antibiotics as the data obtained from the study were statistically significant ($\alpha = 0.05$).

Multiple Antibiotic Resistance (MAR) Indices of the Isolates The study showed that the isolates exhibited varying MAR indices ranging from 0.1 to 0.7 as shown in Table 3. The isolates exhibited MAR indices of 0.1 to 0.7 except isolate R which exhibited MAR index of 0.3, 0.6, and 0.7**.** Isolate Q, R and Y exhibited the MAR index of 0.7 where the maximum degree of resistance was attained in this study. Therefore, the four isolates exhibited high MAR index as their MAR index exceeded 0.2.

Table 1: Conventional antibiotic susceptibility patterns of the isolates

Isolate	N	Susceptibility Strain (%)	Resistance Strain (%)
Isolate Q	58	44 (75.86)	14 (24.14)
Isolate R	4	0 (0.00)	4 (100.00)
Isolate S	12	7 (58.33)	5 (41.67)
Isolate Y	28	17 (60.71)	11 (39.23)
TOTAL	103	68 (66.02)	34 (33.68)

Table 2: Degree of resistance exhibited by the isolates

Isolate	NR	SAR (%)	MAR (%)
SSS10	14	5 (35.71)	9 (64.29)
SS347	4	0 (0.00)	4 (100.00)
SS9401240	5	2 (40.00)	3 (60.00)
SSINT-10	11	4 (36.36)	7 (63.64)
Total	34	11 (32.35)	23 (67.65)

$X^2(2.19) < CV(7.81); \alpha > 0.05$

Table 3: Multiple antibiotic resistance (MAR) indices of th e isolates

Mar Index (%)	Isolate Q n=14	Isolate R (%) n=4	Isolate S (%) n=5	Isolate Y (%) n=11
0.1	5 (35.71)	0 (0.00)	2 (40.00)	4 (36.36)
0.2	1 (7.14)	0 (0.00)	1 (20.00)	0 (0.00)
0.3	3 (21.43)	1 (25.00)	0 (0.00)	2 (18.18)
0.4	0 (0.00)	0 (0.00)	1 (20.00)	0 (0.00)
0.5	1 (7.14)	0 (0.00)	1 (20.00)	3 (27.27)
0.6	3 (21.43)	1 (25.00)	0 (0.00)	1 (9.09)
0.7	1 (7.14)	2 (50.00)	0 (0.00)	1 (9.09)

Discussion

The resistance of different strains of *Streptococcus suis* isolated from the pigs and environs against the conventional antibiotics observed in this study agrees with the findings of many researchers (Palmieri *et al.*, 2011; Huang *et al.*, 2016; Hernandez-Garcia *et al.*, 2017; Libante *et al*, 2019; Yongkiettrakul *et al.*, 2019; Segura *et al.*, 2020). Palmieri *et al.* (2011) reported that resistance to antibiotics could be attributed to the massive use of antibiotics in piggery industries either for growth promotion, prophylaxis or therapy and these attributes to the emergence and spread of antibiotic resistance. They also reported resistance of *Streptococcus suis* against tetracycline, macrolides, aminoglycosides, chloramphenicol, and cadmium salts. The above findings also corroborate with the findings of Hernandez-Garcia *et al.* (2017) and Yongkiettrakul *et al.* (2019) who reported that the recent *S. suis* isolates have become resistant to all classes of antibiotics used in pigs. Isolate R showed 100% resistance to the conventional antibiotics and this confirms the report of Hernandez-Garcia *et al.* (2017) who pointed out that those *S. suis* isolated from non-clinical cases are more resistant than those isolated from clinical cases. Yongkiettrakul *et al.* (2019) reported commensal sites (non-clinical sites) were the sites of transmission of *S. suis* resistance strains to other pigs.

The occurrences of more multiple antibiotic resistance (MAR) strains of *S. suis* than single antibiotic resistance (SAR) strains observed in this study corroborated with the findings of Haung *et al.* (2016), Huang *et al.* (2019) and Yongkietrakul *et al.* (2019), Hernandez-Garcia *et al.* (2017) reported that the existence of multiple antibiotic resistance (MAR) was due to endogenous resistome such as ribosomal protection genes, gene for methylase medicated target site modification and exogenous genetic elements such as integrative and conjunctive elements, transposons, genomic islands phases and chimeric elements.

The varying MAR indices ranging from 0.1-0.7 exhibited by the studied isolates supported the report of Huang *et al.* (2016) who considered *S. suis* as a niche for antimicrobial resistance and represents a high risk of transmission of resistance to other pathogens. Libante *et al.* (2019) made comprehensive research in existence identification of antimicrobial resistance genes present in *S. suis* and found out that high MAR almost occurred in the organism. The occurrences of high MAR index (> 0.2) in this study calls for urgent attention and intervention.

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. The isolates exhibited different degrees of resistance to the conventional antibiotics of which multiple antibiotic resistant (MAR) strains were predominant.

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