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Virulence markers and antimicrobial resistance of *Streptococcus suis* isolated from diseased pigs

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Streptococcus suis causes a wide range of illness in pigs, such as meningitis, septicemia, pneumonia, endocarditis and arthritis (Levesque *et al*. 2016). Muramidase-released protein (MRP), extracellular factor (EF), and suilysin (SLY), encoded by the genes *mrp, epf,* and *sly,* respectively, are considered virulence markers and are used to predict the virulence of a given *S. suis* strain (Espinosa *et al*. 2013). As *S. suis* is found to be associated in various disease conditions in pigs such as arthritis, septicaemia, pericarditis and pneumonia, the present study was undertaken to determine the potential virulence factors and antimicrobial resistance of *S. suis* isolated from pigs with such disease conditions.

S. suis (34) isolated from various disease conditions of pigs (1 to 3 months of age) during the period of February 2011 to January 2016 were used for the study. For isolation of the organism from cases of arthritis and pericarditis, joint fluid and tissues from lesions of pericarditis/pericardial fluid respectively, were cultured. Pieces of lung collected aseptically from dead animals were used for isolation of the organism from lungs. Septicaemic cases were diagnosed by isolating the organism from heart blood. Appropriate amounts of materials from infected organs were aseptically transferred to the surface of a Columbia agar plate supplemented with 5% bovine blood and spread by a sterile triangle. Further dilution of the materials were achieved by using the same triangle on a second and third Columbia agar plate. The plates were incubated aerobically at 37ºC and inspected for growth after 24 and 48 h. Colonies 1–2 mm in diameter showing green or brown decolourization around the colony were suspected as potential *S. suis*. The colonies were confirmed as *S. suis* by both biochemical tests and the *gdh* (glutamate dehydrogenase) -specific PCR amplification product (Okwumabua *et al*. 2003).

DNA template from each isolate was prepared as per Okwumabua *et al*. (2003). Amplification of virulence-associated genes (VAGs) such as suilysin (*sly*), muramidase released protein (*mrp*) and extracellular protein factor (*epf*) including arginine deiminase (*arcA*) was achieved by monoplex PCR assays using the sequence of primers and conditions described previously by Silva *et al.* (2006).

Antimicrobial susceptibility testing was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2008).

Majority (47.05%) of the isolates were from cases of pneumonia (Table 1) which corroborated with the findings of Espinosa *et al*. (2013) who opined that *S. suis* is an invasive pathogen and a very successful colonizer of mucosal surfaces, in particular of the upper respiratory tract. Reports of isolation of this bacterium from pigs suffering from arthritis, pericarditis and septicaemia are also frequent (Levesque *et al*. 2016). The distribution of virulence associated genes is shown in Table 1. *mrp* (Fig. 1) and *arcA* genes were present in 79.41% (each) of the isolates whereas *sly* (Fig. 2) gene was carried by 64.70% isolates in the present study. *mrp*$^+$ + *sly*$^+$ + *arcA*$^+$, *mrp*$^+$ + *sly*$^+$, *mrp*$^+$ + *arcA*$^+$ and *sly*$^+$ + *arcA*$^+$ genes were carried by 23.52%, 20.58%, 35.29% and 20.58% isolates, respectively. Although Espinosa *et al*. (2013) reported detection of *epf* gene in *S. suis* isolates from pigs, all the isolates in the present study were negative for this gene which is consistent with the findings of Zheng *et al*. (2014) who also found that most of the *S. suis* isolates obtained from China were *epf* negative. Interestingly all the invasive strains (strains

Table 1. Virulence markers of *S. suis* isolated from diseased pigs

Virulence associated genes	Number of *S. suis* isolates				Total
	Pericarditis	Arthritis	Septicaemia	Pneumonia	
mrp$^+$ + *sly*$^+$ + *arcA*$^+$	1	4	1	2	8
mrp$^+$ + *sly*$^+$	2	3	1	1	7
mrp$^+$ + *arcA*$^+$	0	0	0	12	12
sly$^+$ + *arcA*$^+$	1	2	3	1	7
Total	4	9	5	16	34

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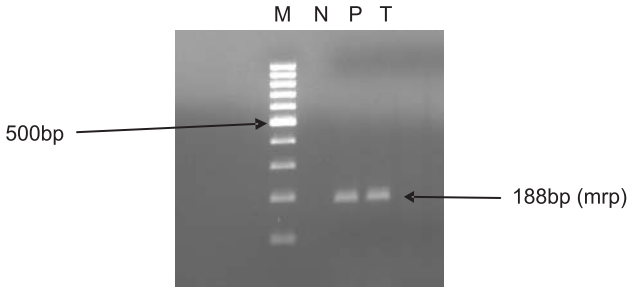

Fig. 1. Detection of *mrp* gene of *S. suis* from pig by PCR. Lane M, Molecular marker (100bp DNA ladder); Lane N, Negative control; Lane P, Positive control; Lane T, Test organism.

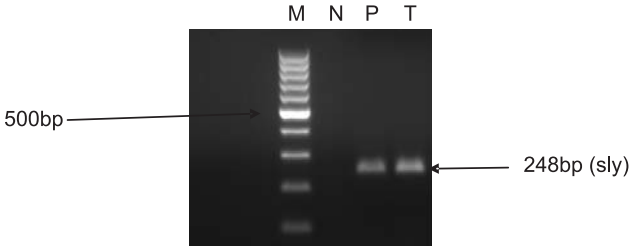

Fig. 2. Detection of *sly* gene of *S. suis* from pig by PCR. Lane M, Molecular marker (100bp DNA ladder); Lane N, Negative control; Lane P, Positive control; Lane T, Test organism.

isolated from cases of arthritis, pericarditis and septicaemia) in the present study carried *sly* genes whereas only 25% (4 out of 16) strains from cases of pneumonia carried this *sly* gene which corroborated with the findings of He *et al*. (2014).

In the absence of effective vaccines to fight against *S. suis*, antimicrobial agents have become increasingly important in treating and controlling the infection of *S. suis*. Of these, 5ØýÞ-lactams, tetracyclines, sulphonamides and macrolides are the most common antimicrobials used for the prevention and treatment of streptococcal infection in pig production. The antibiotic sensitivity pattern of *S. suis* is shown in Table 2. It was observed that the most effective antimicrobial against *S. suis* in the present study was ampicillin (85.29%) followed by penicillin (79.41%). The lowest resistance of *S. suis* isolates for β-lactams was in accordance with the findings of Bojarska *et al.* (2016), supporting their use as a primary drug for treating *S. suis* infections in pigs. For penicillin, this result (lower resistance) may be due in part to the unique mechanism by which resistance is acquired through modifications in the structure of penicillin-binding proteins (Varela *et al*. 2013). We have also observed that *S. suis* isolates in the present study showed high frequency of resistance to tetracycline (82.35%) which is consistent with the reports of Varela *et al*. (2013) who opined that tetracycline resistance among *S. suis* isolates is well documented worldwide. The resistance to tetracyclines in *S. suis* has become a major worldwide problem, closely related to the widespread use of tetracycline in swine production and tetracycline resistance has been considered to be an important cofactor in the selection of resistance to macrolides/lincosamides

Table 2**.** Antimicrobial susceptibility of *S. suis* isolates from pigs (N=34)

Antimicrobials used	Number of *S. suis* isolates		
	Sensitive	Intermediately sensitive	Resistant
Ampicillin	29 (85.29)	3 (8.82)	2 (5.88)
Ciprofloxacin	17 (50.00)	2 (5.88)	15 (44.11)
Enrofloxacin	19 (55.88)	3 (8.82)	12 (35.29)
Erythromycin	11 (32.25)	2 (5.88)	21 (61.76)
Gentamicin	21 (61.76)	3 (6.25)	10 (29.41)
Penicillin	27 (79.41)	2 (5.88)	5 (14.70)
Streptomycin	14 (41.17)	1 (2.94)	19 (55.88)
Sulfadiazine	7 (20.58)	2 (5.88)	25 (73.52)
Tetracycline	4 (11.76)	2 (5.88)	28 (82.35)
Trimethoprim	10 (29.41)	3 (8.82)	21 (61.76)

N, number of isolates tested. Figures in the parentheses indicate the percentage.

(Varela *et al*. 2013). Many antimicrobials used in veterinary practice belong to the same classes as antimicrobials used to treat humans (Weese *et al*. 2015). Use of antimicrobials in livestock may allow transfer of resistant bacteria among animals and resistance genes may be transferred to both commensal organisms and animal or human pathogens (Weese *et al*. 2015). *S. suis* may act as an antibiotic resistance reservoir contributing to the spread of resistance genes to streptococcal human pathogens. Thus, occurrence of relatively high levels of resistance of *S. suis* to some antimicrobials (e.g. macrolides, tetracyclines, and sulphonamides) as observed in the present study may represent a human health concern.

SUMMARY

The study was conducted to determine the virulence associated genes and antimicrobial resistance of *S. suis* associated with various disease conditions of pigs. The VAGs *mrp* and *arcA* were present in 79.41% (each) of the isolates whereas *sly* gene was carried by 64.70% isolates. $mrp^+ + sly^+ + arcA^+$, $mrp^+ + sly^+$, $mrp^+ + arcA^+$ and $sly^+ + arcA^+$ genes were carried by 23.52%, 20.58%, 35.29% and 20.58% isolates respectively. All isolates were negative for *epf* gene. Interestingly all the invasive strains carried *sly* genes whereas only 25% strains from cases of pneumonia carried this gene. The most effective antimicrobial against *S. suis* was ampicillin (85.29%) and the isolates showed high frequency of resistance to tetracycline (82.35%). The occurrence of relatively high levels of resistance of *S. suis* to some antimicrobials (e.g. macrolides, tetracyclines, and sulphonamides) as observed in the present study may represent a human health concern.

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