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Detection of glutamate *dehydrogenase gene* (*gdh*) in *Streptococcus suis* isolated from pigs

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Streptococcus suis infection, a zoonotic disease (Perch *et al*. 1968), causes arthritis, pneumonia, septicaemia, endocarditis, polyserositis, and abscesses in pigs with high economic losses. Subclinical carrier animals are important source as bacteria are transmitted to susceptible young pigs (Okwumabua *et al.* 2003). Detection of *S. suis* by standard culture methods and serotyping are laborious and results can be inconclusive or ambiguous. This study was conducted keeping in view the emergence of *S. suis* infections, to detect the presence of *gdh* gene from *S. suis* isolated from porcine origin by PCR.

Isolates (15) of *Streptococcus suis* were isolated from the 126 samples consisting of nasal swabs of apparently healthy (69) and different clinical samples, viz. heart blood, joint fluid and lungs of diseased (57) pigs from AICRP on Pig, Khanapara and NRC on pig, Rani, Guwahati. The non-*S. suis* isolates, consisting of several Gram-positive bacteria, including streptococcal species and Gram-negative bacteria, were obtained from different clinical samples in the laboratory (Table 1). Identification of all these isolates was confirmed by cultural and biochemical tests as per standard procedures (Facklam 1980, Rosendal *et al*. 1986, Tarradas *et al*. 1994).

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DNA extraction of *S. suis* was done using DNeasy blood and tissue kit following the manufacturer's instruction. PCR was performed to detect *gdh* gene of *S. suis* using oligonucleotide primers (Table 2) according to Okwumabua *et al.* (2003).

The amplification of bacterial DNA for *gdh* gene was performed in thermal cycler in 50µl volume containing 5 µl of 10X PCR buffer, 1µl dNTPs, 1 µl primers, 3 µl of the template DNA and 1µl of Taq DNA polymerase. The conditions for PCR were 94°C for 5 min for initial denaturation of DNA within the sample followed by 30 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (primer annealing), 72°C for 1 min (DNA extension) and a final extension at 72°C for 7 min. The amplified PCR products of desired size were visualized by submarine gel electrophoresis using 4 µl of PCR product on 1.5% agarose gel in 1 X TBE buffer for 75 min at 80V. The amplified DNA fragments of specific sizes were located by Gel Doc System and the image was captured using Alpha Imager EP software after staining with ethidium bromide (0.5µg/ml). Molecular size markers were included in each gel.

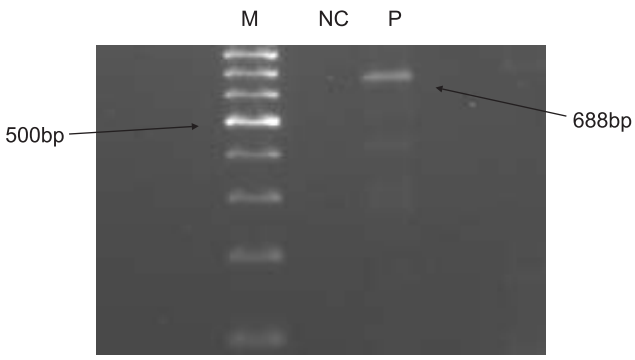
In the present study the PCR primers obtained for *S. suis gdh* gene amplified the DNA from all the 15 *S. suis* strains tested and yielded the expected 688–bp product

Table 1. Non- *S. suis* isolates used in the study

Other streptococcal species (n=5)	Other bacteria (n=5)	Other Gram-positive bacteria (n=5)
Streptococcus dysagalactiae	*Escherichia coli*	*Bacillus subtilis*
Streptococcus zooepidemicus	*Klebsiella pneumonia*	*Clostridium perfringens*
Streptococcus uberis	*Pseudomonas aeruginosa*	*Listeria monocytogenes*
Streptococcus equisimilis	*Salmonella species*	*Staphylococcus aureus*
Streptococcus pyogenes	*Aeromonas hydrophila*	*Enterococcus faecalis*

Table 2. Primes used for amplification of *gdh* gene

Primer	Primer sequence (5′ - 3)	Product size (bp)
JP4 F	GCAGCGTATTCTGTCAAACG	688
JP5 R	CCATGGACAGATAAAGATGG	



M: 100bp plus DNA ladder, NC : Negative control, P: positive sample

Fig. 1. PCR detection of *gdh gene* of *Streptococcus suis.*

(Fig.1) with no false negative, which is in accordance with the results of Okwumabua *et al.* (2003). Although, a polymerase chain reaction was evaluated previously for *S. suis* by Okwumabua *et al.* (1999), the results showed that the targeted DNA regions were not conserved across capsular types or pathogenic strains. Additionally, the presence or lack of targeted genes in several isolates was influenced by geographical location. Creighton (1984) reported that glutamate dehydrogenase (GDH) are highly conserved and exhibited an extremely low rate of point mutation relative to many other genes and was shown by Lyerly *et al.* (1991) to be used successfully in the diagnosis of *Clostridium difficile*. Moreover, Okwumabua *et al.* (2001) showed that analogous to GDH of *C. difficile*, the *S. suis gdh* gene is conserved across *S. suis* capsular types irrespective of geographic origin. As *S. suis gdh*-based PCR assay relates to specificity, DNA from non-*S. suis* bacteria (streptococcal species, n=5; other Gram-positive species, n=5; and other bacteria including Gram-negative species, n=5) were used as the templates for PCR to test the diagnostic efficacy during the present investigation. No amplification product was detected, indicating specificity of the primers. Thus, the present study confirmed that the the *S. suis* isolates can be detected regardless of serotype or geographic origin by targeting the *gdh* gene which are species-specific, highly conserved and thus, is of diagnostic importance.

SUMMARY

The study was undertaken for the detection of *glutamate dehydrogenase gene* (*gdh*) in *S. suis* from pig isolates. The results showed that the PCR primers obtained for *S. suis gdh* gene amplified the DNA from all the 15 *S. suis* strains tested and no amplification product was obtained from the non- *S.suis* isolates. The PCR technique by targeting the *gdh* gene was found highly specific and sensitive and can be used successfully for the detection of *S. suis* isolates regardless of serotype or geographic origin as compared to the conventional biochemical and serological tests, which do not unambiguously differentiate *S. suis* from other related organisms.

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