

## Original Research Article

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## Isolation, Molecular Identification and Antibiogram of *Streptococcus dysgalactiae* Isolates Recovered from Pigs

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### ABSTRACT

*Streptococcus dysgalactiae* is a pyogenic species pathogenic both for animals and humans. It is an emerging pathogen classified as  $\beta$  haemolytic streptococci belonging to Lancefield group C and G. Present study was undertaken to isolate, identify and analyse the antibiogram of *S. dysgalactiae* isolates recovered from pigs. A total of 53 samples were collected from slaughtered pigs, out of which 35 isolates confirmed as Gram positive streptococci microscopically. Biochemically 31 isolates were identified as *Streptococcus dysgalactiae* subspecies *equisimilis* and 4 as group L *Streptococci* by using API STREP 20multitest kit identification system. PCR targeting 23S rDNA gene sequence which confirmed 31 isolates as *Streptococcus dysgalactiae*. Antibiogram of these isolates was done using 13 antibiotic discs for testing antimicrobial sensitivity using standard disc diffusion method. All the isolates were highly sensitive to  $\beta$  lactams followed by fluoroquinolones, tetracyclines, least susceptible to sulphonamides, aminoglycosides and were complete resistant to neomycin. Hence the study highlights the presence of emerging opportunistic pathogen *S. dysgalactiae* in pigs and its decreasing susceptibility towards aminoglycosides. This study is significant as microorganism *Streptococcus dysgalactiae* subsp *equisimilis* is an emerging pathogen in humans as well.

### Keywords

Molecular identification  
Antibiogram,  
*Streptococcus dysgalactiae*, Pigs

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### Introduction

*Streptococcus dysgalactiae* is a gram positive, beta haemolytic cocci belonging to family *Streptococcaceae*. *Streptococcus dysgalactiae* infects humans and as well as animals. *Streptococcus dysgalactiae* is currently divided into the subspecies *Streptococcus dysgalactiae* subspecies *equisimilis* and *Streptococcus dysgalactiae* subspecies

*dysgalactiae*; the former mostly associated with human disease, and the latter almost exclusively encountered in veterinary medicine (VIEIRA *et al.*, 1998).

The clinical manifestations in human disease range from superficial skin-infections and tonsillitis, to severe necrotising fasciitis and bacteraemia (Hughes *et al.*, 2009; Sri *et al.*, 2018).

Animal species are susceptible to infection by *S. dysgalactiae*, but bovine mastitis and infectious arthritis in lambs (joint ill) have been most frequently reported (Whist *et al.*, 2007; Rutherford *et al.*, 2017). In veterinary medicine, retrospective examination of bibliographic data about *Streptococcus dysgalactiae subspecies equisimilis* infection is complicated by the frequent nomenclature changes due to the animal origin of the strains (Preziuso *et al.*, 2010). *S. equisimilis* has been isolated infrequently from placentas from aborted, stillborn, and premature foals (Hong *et al.*, 1993).

Endocarditis and arthritis due to *S. equisimilis* infection have been recently described in swine (Kawata *et al.*, 2003), while the data published before refer to isolation of *S. dysgalactiae* without subspecies distinction (Hong *et al.*, 1993). Furthermore, considering the lack of information, it is also difficult to evaluate if the animals play a role in the maintenance and transmission of this potential zoonotic agent.

A PCR identification system targeting 23S rDNA sequences for the identification of eight streptococcal species relevant to animal infections (*S. agalactiae*, *S. bovis*, *S. canis*, *S. dysgalactiae*, *S. equi*, *S. porcinus*, *S. suis* and *S. uberis*) was used in the present study (Kawata *et al.*, 2004).

The aim of present study was identification of isolates recovered from pigs by biochemical and molecular method of identification followed by antibiogram.

## Materials and Methods

### Sample collection

Total 53 samples were collected from pigs which were slaughtered at slaughter house of Bareilly. Heart blood was collected in sterile

vacutainer. Lymph nodes and joint fluid samples were also taken.

### Sample processing

Samples were inoculated in BHI broth (Difco) and kept for incubation at 37°C for 4-6 hours. Loopful of culture was then streaked on 5% sheep blood agar. After overnight incubation under micro-aerophilic condition cultural characteristics were observed. Isolates which showed  $\beta$ -haemolytic colonies (showing complete haemolysis) were picked up and further subcultured on blood agar.

### Culture identification

Cultural characteristics were observed followed by microscopic morphology was observed by Gram's staining method.

### Biochemical characterization

Bacterial isolates were subjected to catalase test and oxidase test. All catalase negative isolates were further screened by API 20 STREP (bioMerieux) multitest kit identification system as per manufacturer's instruction.

### Molecular identification

DNA isolation was done by snap chilled method. Quality and purity of DNA was determined by taking absorbance at 260 and 280nm in UV spectrophotometer. A PCR identification system targeting 23S rDNA sequences (Kawata *et al.*, 2004) was used to screen the recovered isolates.

### Antibiotic sensitivity testing

Antibiotic sensitivity assay of characterized isolates was performed as per CLSI guidelines with slight modifications. Colony suspension, equivalent to a 0.5 McFarland

standard, prepared using colonies from an overnight (18 to 20 hr) sheep blood agar plate at 37 °C. Disk diffusion method was used on MHA with 5% sheep blood.

Zone of inhibition was measured after 18-20 hrs of incubation at 37 °C under micro-aerophilic condition.

## Results and Discussion

### Culture identification

Out of 53 samples 35 pure isolates were obtained which showed clear  $\beta$  haemolysis on blood agar. Colonies were round, whitish to

grey, 1-2 mm in size, raised with entire edge. Microscopically Gram positive cocci present in pairs and chain were seen (Fig. 1 and 2).

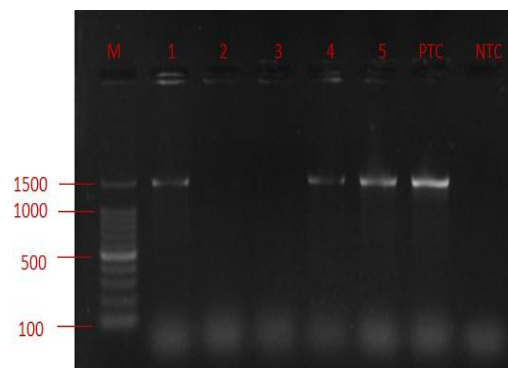
### Biochemical characterization

All the isolates were oxidase and catalase negative. 31 Isolates were biochemically characterized as *Streptococcus dysgalactiae* subspecies *sequisimilis* and 4 isolates as L group streptococci by API 20 STREP mutitiest kit identification system (BioMerieux). Specific number obtained for each isolate which used to identify isolate by apiweb software.

**Fig.1** API kit result after 24 hours of incubation, colour changes in reagents observed for individual cupule



**Fig.2** PCR product of 23S rDNA gene of field isolates confirmed as *S. dysgalactiae*



Lane M: 100bp DNA ladder

Lane 1-5: Amplified PCR product of field isolates, product size 1508 (~1500 bp)

Lane PTC: Positive template control (Field isolate of *S. dysgalactiae* maintained at division of Biological Standardization, IVRI)

Lane NTC: Negative template control

### Molecular identification

Out of 35 isolates 31 isolates were identified as *Streptococcus dysgalactiae* after screening

through reaction A and B as described by Kwata *et al.*, (2004). A product size of 1508 bp was amplified for *S. dysgalactiae*. DNA of the rest of the 4 isolates were not amplified in

either of the reaction, therefore could not be identified.

### Antibiotic sensitivity

All isolates were highly sensitive to  $\beta$  lactam antibiotics, among which wide zone of inhibition was seen for ampicillin and cloxacillin. Resistance was shown by 18 isolates for streptomycin and 24 isolates were resistant to amikacin 80% isolates showed resistance for sulphadiazine and all the isolates were resistant for neomycin.

*S. dysgalactiae* can infect several animal species whereas, *Streptococcus dysgalactiae subspecies equisimilis* can infect humans as well as animals. Currently, bacterial culture and biochemical identification with commercial kits are widely used for *S. dysgalactiae* diagnosis. Isolated  $\beta$ -haemolytic colonies were obtained on 5% sheep blood agar which was a parameter of presumptive identification (Baeck *et al.*, 2006; Ciszewski *et al.*, 2016). 31 isolates were confirmed as *Streptococcus dysgalactiae subspecies equisimilis* by using API 20 STREP multitest kit identification system (BioMerieux), This API multitest kit system has been used earlier by various workers for the biochemical characterization of *S. dysgalactiae* (Poutrel *et al.*, 1984; Baeck *et al.*, 2006). Nevertheless, overgrowing of other beta-haemolytic streptococci or mistakes in biochemical profile interpretation may be encountered during bacterial examination. PCR is widely used in many fields of microbiology.

In PCR *S.dysgalactiae* was identified successfully as described by Kwata *et al.*, (2004) A product size of 1508 bp was obtained.

The isolates of *S. dysgalactiae* were susceptible to  $\beta$ -lactams and this finding was similar to the previous studies (Tsi *et al.*,

2014; Ciszewski *et al.*, 2016; Lu *et al.*, 2016). However resistance was seen for streptomycin, amikacin and sulphadiazine in increasing order while complete resistance was seen for neomycin.

In conclusion the isolates characterized as *S. dysgalactiae* by PCR system. Same isolates characterized as *S. dysgalactiae* subsp. *equisimilis* by API multitest kit identification system. Antibiogram revealed decreasing sensitivity for aminoglycosides. Resistant to commonly used antibiotic azithromycin in upper respiratory tract infection was observed.

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