SHORT COMMUNICATIONS

Isolation of *Streptococcus suis* Using a Selective Medium

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ABSTRACT

A selective medium containing tryptic soy agar, 5% defibrinated bovine blood, crystal violet, nalidixic acid and gentamicin significantly improved the isolation rate of *Streptococcus suis* from tonsilar tissue of slaughtered pigs. Ninety-five percent of the *S. suis* isolates identified in Guelph were confirmed as *S. suis* in Copenhagen, but only six out of 21 isolates typed as capsular serotype 2 in Guelph were confirmed to possess serotype 2 antigen in Copenhagen. Sixty-four percent of the *S. suis* isolates were not typable within the current scheme of capsular serotypes from 1 to 13 and type 1/2.

Key words: *Streptococcus suis*, swine, isolation, selective medium

RÉSUMÉ

L'utilisation d'un milieu sélectif qui contenait une gélose tryptique de soya, 5% de sang bovin défibriné, du cristal violet, de l'acide nalidixique et de la gentamycine, améliora significativement le taux d'isolement de *Streptococcus suis*, à partir d'amygdales de porcs d'abattage. Un laboratoire de Copenhague confirma comme tels 95% des isolats de *S. suis* identifiés à Guelph, mais seulement six des 21 chez lesquels on croyait avoir reconnu l'antigène capsulaire du sérotype #2. Il s'avéra impossible de typer 64% des isolats de *S. suis*, à l'aide du schéma courant des antigènes capsulaires des sérotypes #1 à #13 et du type #1/2.

Mots clés: *Streptococcus suis*, porcs, isolement, milieu sélectif.

Nonenterococcal streptococci of Lancefield's Group D isolated from pigs are usually referred to as *Streptococcus suis* (1,2). Although apparently worldwide in distribution, it was not until the last two decades that they have been recognized as significant pathogens causing such conditions as septicemia, meningitis, pneumonia, arthritis and endocarditis (3). These diseases may occur as outbreaks with high morbidity and mortality generally in pigs between two and 20 weeks of age (4,5). The transmission of the infection is thought to be by healthy carriers harboring the organism in the tonsillar tissue (6).

In addition to group D specific antigen *S. suis* strains possess serotype specific capsular polysaccharides. Strains with serotype 2 capsules are also significant human pathogens resulting in septicemia and meningitis (7). Cases have been reported from Europe, the Far East and Canada (4,7,8). In most, if not all, human cases contact with pigs or pork products have been identified as the most likely source of infection, and the organism is believed to gain entrance through cuts or abrasions in the skin (7) or through the digestive tract (9).

Streptococcus suis grows on blood or serum-enriched media with mucoid colonies. The hemolysis on bovine blood agar is of the α-type, but may turn β on prolonged incubation (10). The bacteria are typically elongated (lancet-shaped) and often arranged in pairs in the Gram-stained smears. Because it is difficult to distinguish *S. suis* colonies from other streptococci on nonselective media, and because *S. suis* resembles *S. pneumoniae* in some cultural and morphological respects (9), we decided to evaluate a selective medium originally recommended for *S. pneumoniae* (Pneumococcus Selectatabs). Such a medium would be useful in epidemiological studies on the prevalence of *S. suis* in the tonsils of slaughtered pigs and studies on exposure risk of humans in contact with pigs or pork products.

Six Pneumococcus Selectatabs® (Mast Lab. Ltd., Mast House, Derby Road, Bootle, Merseyside, UK) were added to one liter of melted tryptic soy

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agar with 5% defibrinated calf blood. Each tablet contained 0.2 mg crystal violet, 5 mg nalidixic acid and 0.2 mg gentamicin. The recommended concentration for media selective for *S. pneumoniae* was ten tablets per liter, but preliminary studies using laboratory strains of *S. suis* indicated that only pinpoint colonies developed during two days' incubation on plates with ten tablets per liter. The size of colonies on plates with six tablets per liter was not different from the size on nonselective plates.

For evaluation of the media 307 samples were collected from palatine tonsils of slaughtered pigs using sterile cotton wool swabs rubbed against a fresh cut surface. The swabs were immediately placed in sterile tryptone broth with 0.02% Na-azide and transported to the laboratory at ambient temperature.

The swabs were streaked onto selective and nonselective blood agar plates within two hours and the plates were incubated for two days at 37° C in normal atmosphere enriched with 5% CO_2. Both selective and nonselective plates were examined for "typical" *S. suis* colonies: small, grayish or transparent, mucoid, α-hemolytic, or α to β hemolytic colonies. Single colonies were transferred to nonselective blood agar plates and incubated overnight.

If examination of pure cultures of colonies picked as "typical" *S. suis* gave the following results, the isolate was identified as *S. suis*: Gram-positive, cocci (mostly elongated or lancet-shaped) in pairs or short chains, catalase negative, inulin positive, glycogen positive, arginine positive (12) and agglutination with Lancefield group D serum (Streptex® kit, Wellcome Diagnostics, 3030 Cornwallis Road, Research Triangle Park, North Carolina). The strains were further tested for agglutinability with *Staphylococcus aureus*, strain Cowan, sensitized with *S. suis* serotype 2 antiserum raised in rabbits (11). A preparation incorporating normal rabbit serum was used as negative control reagent.

In order to validate the results 100 randomly selected isolates identified as *S. suis* were shipped to the Streptococcus Department at the Statens Seruminstitut in Copenhagen, Denmark. These strains were examined independently using procedures described (3,12).

TABLE I. Isolation Rate of *Streptococcus suis* from 307 Tonsillar Samples Streaked on Selective and Nonselective Calf Blood Agar

	"Typical" *S. suis* Colonies	Actual *S. suis*	Percent *S. suis* Identified
Nonselective calf blood agar	137	41	30%
Selective calf blood agar[a]	211	112	53%

[a] 1.2 mg crystal violet, 30 mg nalidixic acid and 1.2 mg gentamicin per liter

A larger number of "typical", *S. suis* colonies was recognized on selective plates than nonselective plates (Table I) and a larger proportion of the colonies picked from selective plates were identified as actual *S. suis* (Table I). The difference was statistically significant (χ^2; $P < 0.01$). The selective medium designed for *S. pneumoniae* therefore appeared to be suitable for *S. suis* except for the concentration of the selective principles which had to be reduced to 60% of the concentration recommended for *S. pneumoniae*. Previously described selective media for *S. suis* may be just as effective (6), but ours is very simple to make up since the inhibitory components are commercially available in concentrations ready to use. The reason for the poorer performance of the nonselective medium was overgrowth with contaminants. The bacteria growing on five randomly selected nonselective plates and the parallel selective plates were identified. The nonselective plates had *Aerococcus* spp., *Pediococcus* spp., *Staphylococcus* spp., α and β-hemolytic *Streptococcus* spp., *Nocardia* spp., *Bacillus* spp., *Pasteurella* spp., *Escherichia coli* and *Proteus* spp. The selective plates had fewer colonies primarily of α and β-hemolytic *Streptococcus* spp., *Staphylococcus* spp. and bacteria of the genus *Gemella*.

Of the 100 randomly selected isolates of *S. suis* 21 reacted with the coagglutination reagent for capsular type 2. When these 100 isolates were sent to Copenhagen for verification, 95 were confirmed biochemically as *S. suis*, but only six of the 21 type 2 strains were confirmed as either type 2 or type 1/2. However, all the isolates typed as 2 or 1/2 in Copenhagen were previously identified as 2 in Guelph. The working definition of *S. suis* suggested by Perch *et al* (12) (glycogen, inulin and arginine positive nonenterococcal group D streptococci) therefore appears to be satisfactory. The reason for the discrepancy in capsular serotyping may be differences in interpretation, use of different tests, or loss of specific capsular antigens during *in vitro* culturing (3). It appears, therefore, that studies should be undertaken to improve consistency of serotyping of *S. suis*. A total of 31 strains were typable within the current scheme covering serotypes 1 through 13 and 64 isolates were nontypable. It is therefore possible that many more serotypes exist than the current serotyping scheme indicates. It is also conceivable that isolates from clinical or pathological material primarily belong to the recognized serotypes because these isolates have attracted most interest, whereas isolates from healthy pigs are mainly nontypable.

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