

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *STREPTOCOCCUS SUI*S ISOLATES TO COMMON ANTIBIOTICS USED IN PIG FARMS

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Invited paper

Abstract: The goal of this study was to determine antimicrobial susceptibility of 34 *Streptococcus suis* strains isolated from healthy and diseased pigs from two pig farms. Disk diffusion was the method used to assess antibacterial susceptibility of *S. suis* according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST standard protocols. Overnight cultures of *S. suis* grown on Colombia 5% blood agar (HIMEDIA, India) were suspended in Todd Hewitt broth (Oxoid Limited, England) and suspension was adjusted to 0.5 McFarland standard. Using sterile swabs suspension was spreaded to Mueller Hinton agar supplemented with 5% defibrinated sheep blood, after which standardized discs containing antimicrobial substances were placed on the agar surface. Plates were incubated at 37 °C in aerobic conditions usually for 18 hours, but no more than 24 hours after which diameter of *S. suis* inhibition zones were measured. Among 34 isolated *S. suis* strains all were resistant to tetracycline (TET), lincomycin (L) and clindamycin (CC). Susceptibility was very low to sulfamethoxazole-trimethoprim (SXT) combination (94.1% resistance), 94.1% of strains were sensitive to penicillin G (P), amoxicillin (AMX), erythromycin (E), enrofloxacin (ENR) and azithromycin (AZM) while sensitivity to cephalosporins of the first (cephalexin-CN), third (cefotaxime – CTX, ceftriaxone - CRO) and fourth (cefepime-FEP) generation, as well as the sensitivity to florfenicol (FFC) and vancomycin (VAN) was absolute (100%). Therefore we can conclude that cephalosporins are very reasonable choice in treatment of human cases of *S. suis* meningitis but also as a treatment of choice for the *S. suis* pig septicemia.

Key words: *Streptococcus suis*, antimicrobial susceptibility, pig, antibiotics

Introduction

Streptococcus suis is Gram positive bacterium that causes great economic losses to the pork industry. *Streptococcus suis* is the major cause of swine meningitis and septicemia, but also it can be a cause of many other pathological conditions such as arthritis, endocarditis, pneumonia, polyserositis, abortions and abscesses and sudden death (Stanojkovic et al., 2017). Except from being a major domestic swine (*Sus domestica*) pathogen, *S. suis* can be often isolated from variety of other mammalian species and birds. Gottschalk et al. (2010) therefore suggest the existence of complex epidemiological patterns of the infection, since other animal species might also be a source of swine infection (Gottschalk et al., 2010). In Western countries human *S. suis* infections have mainly been considered sporadic but in Asian countries this pathogen is one of the most important cause of meningitis (Arends and Zanen, 1988).

Using coagglutination reaction based on polysaccharide capsules method, thirty-five serotypes (serotype 1 to 34 and serotype 1/2) were previously recognized among of *S. suis* species (Messier et al., 2008). Later, according to DNA based methods serotypes 20, 22, 26, 32, 33, and 34 were reclassified and do not anymore belong to *S. suis* species. Recently, 9 novel serotypes (non-typeable strains) have been identified using DNA sequencing, and at this moment *S. suis* species comprises of 38 serotypes. *Streptococcus suis* serotype 2 is the most frequent serotype causing the disease in both, pigs and humans, but also some other serotypes such as 7, 9, 14, 16, 21 can be involved in the pathologic conditions.

Antimicrobial treatment against infection caused by *S. suis* depends on few criteria's such as bacteria antimicrobial sensitivity, type of infection and the application of the antimicrobials. According to Kataoka et al. (2000), Han et al. (2001) and Marie et al. (2002) high sensitivity to penicillin has been established. However, using MIC method it is shown that that penicillin sensitivity is actually intermediate, while amoxicillin and ampicillin sensitivity is around 90%. So it is suggested that penicillin should be used as a treatment only in the cases of determined susceptibility to this drug.

Antimicrobial resistance of *S. suis* strains to some antibacterial medicines such as tetracycline, clindamycin, erythromycin, kanamycin, neomycin and streptomycin has already been described, while the resistance to trimetprim-sulfametoxazole has been very variable. In France, high susceptibility of *S. suis* is shown to be to penicillin, amoxicillin, ceftiofur and gentamicin and very low to lincomycin (38%) and tetracycline (18%) (Marie et al., 2002). In Spain it has been determined (Vela et al., 2005) that over 89% of *S. Suis* strains has been susceptible to penicillin, amoxicillin, ceftiofur, gentamycin, spectinomycin i enrofloxacin while over 87% of strains is resistant to tetracycline, clindamicine and

erythromycin which is attributed to mass use of these medicines in the treatment and disease prevention.

Similar results were obtained in Denmark by *Aarestrup et al. (1998)*. These authors find high resistance to tetracycline and tylosine, high susceptibility to penicillin, ampicilline, ceftiofur and vancomycine, and also variable resistance to macrolide antimicrobials and sulfamethoxazole-trimethoprim combination. *Tian et al. (2004)* six years later find that situation in Denmark has not changed, *S. suis* strains were still susceptible to florfenicol, chloramfenicol, penicilline, ciprofloxacin, sulfamethoxazole and trimethoprim, while high resistance frequency is noted in erythromycin (40.78%) and tetracycline (24.27%).

Wisselink et al. (2006) have investigated *S. suis* susceptibility to different antibacterials currently in use in most of the European countries. In this research all of the strains have been sensitive to enrofloxacin, ceftiofur, cefquinome, florfenicol and penicillin, 1.3% were resistant to gentamicin, 6% were resistant to sulfamethoxazole-trimethoprim combination, while 75.1% were resistant to tetracyclines. Contrary to this, *Tarradas et al. (1994)* in Spain find high resistance to penicillines. Low sensitivity to penicillin (50% of resistant strains) have also been established by *Seol et al. (1996)*, adding that sensitivity to doxycycline was 30%, resistance to amoxicilline, erytromicine and cefalexin was 36.4%, 9.1% and 15% respectively.

In Asia β -lactam antibacterials are still the treatment of choice for *S. suis* infection. Survey in China has showed high resistance to eritromycine, clindamycin, tilmicosine and sulfamethoxazole-trimethoprim (*Zhang et al., 2008*). Ten year survey in Japan which included 689 *S. suis* isolates (*Kataoka et al., 2000*), showed that this bacteria is susceptible to penicilline, cefazoline, ofloxacin and sulfamethoxazole-trimethoprim, while 87% was resistant to tetracyclines, 71.4% was resistant to kanamycin and 29.5% was resistant to streptomycine. In Thailand (*Yongkiettrakul et al., 2019*) it was summarized that *S. suis* strains have high level of antimicrobial susceptibility to vancomycine (96.6%), cefotaxime (93.1%), ceftiofur (94.7%) and florfenicol (92.4%), intermediate level of antibiotic susceptibility to penicillin (33.2%), gentamicin (23.3%), enrofloxacin (21.4%), norfloxacin (27.9%) and sulfamethoxazole-trimethoprim (36.3%) and very low level of antibiotic sensitivity to clindamycin (6.5%), doxycycline (9.2%), tetracycline (5.0%) and tiamuline (2.3%).

Overall, in most of the world countries it is shown that *S. suis* strains are usually resistant in high level to macrolides, lincosamides and tetracycline, variable resistant to aminoglycosides and sulfonamide-trimethoprim combinations, while the resistance to β -lactames, vancomycine, florfenicol, enrofloxacin and ciprofloxacin is usually very low.

Most of the *Streptococcus suis* antimicrobial resistance genes have been identified. Among these, best known and characterized are genes and mechanisms of resistance to macrolides (ermB), tetracyclines resistance (tetM and tetO), penicillins (PBP 1, PBP 2 and PBP 3), fluorohinolones (point mutations) and chloramfenicol (transpozons).

Materials and Methods

Antimicrobial susceptibility was carried out on 34 *Streptococcus suis* strains isolated from healthy and diseased pigs from two pig farms. Method for isolation and identification of *S. suis* isolates was the one described by *Stanojkovic et al. (2015)*. Disk diffusion was the method used to assess antibacterial susceptibility of *S. suis* according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST standard protocols. Overnight cultures of *S. suis* grown on Colombia 5% blood agar (HIMEDIA, India) were suspended in Todd Hewitt broth (Oxoid Limited, England) and suspension was adjusted to 0,5 McFarland standard. Using sterile swabs suspension was spreaded to Mueller Hinton agar supplemented with 5% defibrinated sheep blood, after which standardized discs containing antimicrobial substances were placed on the agar surface. Plates were incubated at 37 °C in aerobic conditions usually for 18 hours but no more than 24 hours after which diameter of *S. suis* inhibition zones were measured.

Antibiotic discs used in the survey were the ones often used in pig farms such as penicillin G (P), amoxicillin (AMX), tetracycline (TET), sulfamethoxazole/trimethoprim (SXT), lincomycin (L), erythromycin (E), enrofloxacin (ENR), florfenicol (FFC), cephalexin (CN), but also some used in treatment of human streptococcal infections such as clindamycin (CC), azithromycin (AZM), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP) and vancomycin (VAN).

Results and Discussion

Results of antimicrobial susceptibility testing of 34 *Streptococcus suis* isolates are presented in table 1 and table 2 (percentage values).

Table 1. Number of resistant and sensitive isolates of *Streptococcus suis*

<i>Streptococcus suis</i>	Susceptibility	ANTIBACTERIALS														
		P	Amx	Te	Sxt	L	E	Enr	Ffc	Cn	Cc	Azm	Ctx	Cro	Fep	Va
R		2	2	34	32	34	2	2	0	0	34	2	0	0	0	0
S		32	32	0	2	0	32	32	34	34	0	32	34	34	34	34

*R –Resistant; S –Sensitive

Table 2. Percentage of resistant and sensitive isolates of *Streptococcus suis*

<i>Streptococcus suis</i>	Susceptibility	ANTIBACTERIALS														
		P	Amx	Te	Sxt	L	E	Enr	Ffc	Cn	Cc	Azm	Ctx	Cro	Fep	Va
R		5.9	5.9	100	94.1	100	5.9	5.9	0	0	100	5.9	0	0	0	0
S		94.1	94.1	0	5.9	0	94.1	94.1	100	100	0	94.1	100	100	100	100

*R –Resistant; S –Sensitive

Among 34 isolated *S. suis* strains all were resistant to tetracycline (TET), lincomycin (L) and clindamycin (CC). This corresponds to data provided by Vela et al. (2005) who determined that more than 87% of *S. suis* strains are resistant to tetracycline and clindamycin, as well as the most data collected in European countries (Marie et al., 2002; Aarestrup et al., 1998; Wisselink et al., 2006) that suggest that resistance to tetracyclines, lincomycine and clindamycin is already very high or sometimes total. Same results about low tetracyclines and clindamycin sensitivity were also reported in China by Zhang et al. (2008) in Japan (Kataoka et al., 2000) and Thailand (Yongkiettrakul et al., 2019).

Susceptibility to sulfamethoxazole-trimethoprim (SXT) combination was very low in this research (94.1% resistance). This result was opposite to the result obtained in Denmark (Tian et al., 2004) and most EU countries (Wisselink et al., 2006) which show high sensitivity rate of *S. suis* to this antibacterials. Regarding Asian countries, this result is in compliance to results in China and Thailand but opposite to the one obtained in Japan where sulfamethoxazole-trimethoprim has good activity against *S. suis*.

Explanation for the very high resistance to tetracycline (TET), lincomycin (L) and sulfamethoxazole-trimethoprim (SXT) combination lies in the

fact that these antibacterials were so far the most used drugs in pig farms in „controlled” prevention of different pig diseases. Over the time *S. suis* strains, like many other bacteria became resistant to these antimicrobials.

Like in the most European countries high sensitivity (94.1%) to penicillin G (P), amoxicillin (AMX), erythromycin (E), enrofloxacin (ENR) and azithromycin (AZM) has been found in this research. Unfortunately there are also data that suggest that it might be high or intermediate sometimes (Tarradas *et al.*, 1994; Yongkiettrakul *et al.*, 2019). Resistance to erythromycin and azithromycin usually depends on the country or country area, or sometimes on the specificity of the farm in the survey, and it varies from low to intermediate or very high.

Sensitivity to cephalosporins of the first (cephalexin-CN), third (cefotaxime – CTX, ceftriaxone - CRO) and fourth (cefepime-FEP) generation, as well as the sensitivity to florfenicol (FFC) and vancomycin (VAN) was absolute (100%). Although sensitivity to florfenicol is 100% it can be expected that in the near future resistant strains arise because of the more frequent use of this antibacterial in Serbian pig farms. Cases like this are reported in countries where florfenicol is often used to treat pig respiratory infections (Zhang *et al.*, 2008).

High cephalosporin sensitivity is of importance for the human cases of *S. suis* infection. Human cases of *S. suis* infection are usually acquired through close contact with pigs or pig meat, so strains infecting humans usually have same susceptibility to antimicrobials. Reports from human cases of *S. suis* meningitis (Wangkaew *et al.*, 2006; Lun *et al.*, 2007) show successful empirical use of third generation cephalosporins in the treatment of the disease caused by this pathogen. Cephalosporins are known to have good penetration into the brain tissue, crossing the blood-brain barrier, have a broad spectrum antimicrobial activity, and like in this research it is shown to have excellent activity against *Streptococcus suis*.

Regarding results in this research we acknowledge that results of antimicrobial susceptibility testing are generally similar to the results other authors find in European countries. Differences can be found not only between countries but sometimes even among pig farms in the same country. Therefore, problem of antimicrobial susceptibility of *Streptococcus suis* in one country must be approached on the systemic micro level, from one to another pig farm.

Conclusion

The results of this study regarding antimicrobial susceptibility testing showed that *Streptococcus suis* strains in Republic of Serbia are most susceptible to cephalosporin antibiotics, but also to florfenicol and vancomycin. High

susceptibility was to penicillin, amoxicillin, erythromycin, enrofloxacin and azithromycin, very low to sulfonamide-trimethoprim combination and all strains of *S. suis* were resistant to tetracycline, lincomycin and clindamycin. Because cephalosporins have a good penetration into the brain tissue and broad spectrum antimicrobial activity, we can conclude that cephalosporins are very reasonable choice in treatment of human cases of *S. suis* meningitis but also as a treatment of choice for the *S. suis* pig septicemia.

Antimikrobna osetljivost izolata *Streptococcus suis* na antibiotike koji se najčešće koriste na farmama svinja

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Rezime

Cilj ovog ispitivanja je bio da se odredi antimikrobna osetljivost 34 soja *Streptococcus suis* izolovanih od bolesnih i zdravih svinja sa dve farme svinja. Disk difuziona metoda je korišćena u ispitivanju osetljivosti prema standardima opisanim od strane CLSI i EUCAST. Kulture *S. suis* dobijene na Kolumbija agaru sa 5% ovčije krvi su suspendovane u Todd Hewitt bujon i zamućenje je centrirano na 0,5 jedinica McFarland standarda. Sterilnim štapićem za bris je suspenzija razmazana po Mueller Hinton agaru sa dodatkom 5% defibrinisane ovčije krvi nakon čega su na površinu agara postavljeni diskovi sa antimikrobnim supstancama i agari inkubirani na 37 °C u aerobnim uslovima 18 časova i ne duže od 24 časa. Nakon inkubacije merene su zone inhibicije oko diskova. Svi sojevi *S. suis* su bili otporni na dejstvo tetraciklina, linkomicina i klindamicina, dok je 94,1% bilo otporno na kombinaciju sulfometoksazola i trimetoprima. Nasuprot tome, 94,1% je bilo osetljivo na dejstvo penicilina G, amoksicilina, ertromicina, enrofloksacinai azitromicina, dok su svi sojevi bili osetljivi na dejstvo cefalosporina prve generacije cefaleksina, treće generacije cefotaksima i ceftriaksona i četvrte generacije cefepima, ali i na dejstvo florfenikola i vankomicina. Stoga se može zaključiti da su cefalosporini razuman izbor empirijske terapije meningitisa ljudi koji izaziva *S. suis*, kao i tretman izbora septikemije svinja koju izaziva ova bakterija.

Ključne reči: *Streptococcus suis*, antimikrobna osetljivost, svinje, antibiotici