



Skin Bacteriome and its Resistance to Antibiotics in Free Range Pigs

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RESEARCH ARTICLE

Abstract

The cutaneous microbiome and also its resistance to antibiotics is exposed to change, depending on different habitat factors. This research investigates the composition of cutaneous microflora and its antibiotic resistance in pigs raised on free range farms (mix breed swine, from low input small farms). Swabs were collected from the skin surface and subjected to classical microbiological methods (simple broth and nutrient agar cultivation, colony isolation and biochemical API identification). The antibacterial resistance to gentamicin, streptomycin, oxitetracycline, tylosin, amoxicillin-clavulanic acid, marbofloxacin, tulatromycin, cefotaxime and doxycycline was estimated by Kirby Bauer method and multiple antibiotic resistance (MAR) index was calculated. Strains from *Staphylococcus* (*sciuri* and *warnerii*), *Shigella* spp., *Kytococcus sedentarius*, *Salmonella* spp. and *Citrobacter freundii* genera and species were identified in the collected samples. The most resistant was a *S. warnerii* strain, but the MAR index was high (0.33) in 50% of the strains. The most efficient antibiotic was cefotaxime and the least efficient was oxitetracycline. The results indicated the presence of antibiotic resistant ubiquitous and pathogenic strains in the investigated pigs which need caution, since they could express pathogenicity under appropriate conditions which low input farming system could provide.

Keywords: swine, free range, skin bacteriome, antibiotic resistance.

INTRODUCTION

Low input, small pig farms are traditional Romanian farming systems. In this type of farms, the cutaneous microbiome is exposed to environmental factors and could suffer major changes, such as changes in bacteria genera and species and their resistance to antibiotics (Popescu, 2013; Curtis et al., 1975). Considering the close connection between the environment and swine shelters, there are higher chances for the animals to contract various diseases or to create propitious background for bacteria present on the skin to grow and induce pathological conditions. Such pathologies may cause major economic consequences (Popescu, 2013). Besides the close environment-shelter connection that appears in this housing system, another close interaction, the one between humans and pigs is also relevant. This might increase the possibilities of humans getting infected with zoonotic agents via cutaneous contact (livestock associated meticillin resistant *Staphylococcus aureus* - LA-MRSA, erysipelotrix, etc.) (Strube et al., 2018;

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Sriskandan and Slater, 2006). Studies show that each individual has a typical skin microflora, which is closely related to the habitat conditions and to the sow-piglet contact during first days of life. Bacteria found on swine skin are divided in 4 phyla: phylum *Firmicutes* (mostly Gram positive bacteria), classes *Bacilli*, *Clostridia*, *Erysipelotrichia*, *Negativicutes*, *Thermolithobacteria*; phylum *Bacteroidetes*, classes *Rhodothermia*, *Balneolia*, *Cytophagia*, *Sphingobacteria*, *Chitinophagia*, *Bacteroida*, *Flavobacteriia*; phylum *Actinobacteria*, classes *Rubrobacteria*, *Thermoleophilia*, *Coriobacteriia*, *Acidimicrobiia*, *Nitriliruptoria*, *Actinobacteria* and phylum *Proteobacteria*, classes *Alphaproteobacteria*, *Betaproteobacteria*, *Hydrogenophilalia*, *Gammaproteobacteria*, *Acidithiobacillia*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Oligoflexia*. Of all these, the most commonly identified strains from skin samples are bacteria from genera *Staphylococcus*, *Streptococcus* and *Micrococcus* (McIntyre et al., 2016).

The importance of skin bacteriome studies reside in its protective role, but also in its possible association with disease or health (Švejtil et al., 2018). Similarly, the skin bacteriome represents a valid model for study trials (transposition to human health studies)(Bush et al., 1986). Research on the antimicrobial resistance of the skin bacteriome can provide valuable information about the beneficial or unwanted influence of various environmental factors on bacteria and changes they might suffer (increased virulence, increased pathogenicity, potential antibiotic resistance gene transfer, etc.). Further, such investigations allow a better insight to a more fit therapeutic approach and better designed therapeutic protocols (McIntyre et al., 2016; Nowland et al., 2019) to protect the host but also the environment within the „One health” framework.

This research aimed to investigate the composition of cutaneous microflora by bacterial identification and characterization as well as its antibiotic resistance in pigs raised on low input, free range farms.

MATERIALS AND METHODS

The research was conducted on pigs (n=10) from mixed breeds, raised on low input small farms (Figure 1). Both farms had mixed domestic animal population in contact with the pigs: on one of the farms, pigs contacted with chickens, while on the other, pigs were in close connection with goats. Both the chickens and the goats were accommodated on the farm, in adequate paddocks, without access to a pasture.



Figure 1. Low input, small pig farms: typical swine shelters in Romania villages

Swabs were collected from the skin surface and subjected to classical microbiological methods and identification.

The samples were processed by the classical bacteriology method. Each swab was immersed for seeding in a tube with simple broth. The tubes were subjected to incubation for 24 h, at a temperature of 37°C. Further, the obtained cultures were inseeded on Mueller-Hinton agar in sterile plastic Petri dishes, by use of a sterile loop.

The plates were placed in an incubator at 37°C for 24 h.

After cultivation, each colony was characterized macroscopically and smears were stained by Gram stain. The shape and G+/G- staining of isolated bacteria were recorded.

For strain identification, a biochemical method was used. The Remel RapID™ test kits represented a qualitative micromethod of identification, based on differentiated degradation of specific substrate by different bacteria (Glover et al., 2017).

The antimicrobial resistance was estimated to six groups of antibiotics, such as: aminoglycosides (gentamicin, streptomycin), tetracyclines (oxitetracycline, doxycycline), penicillines (amoxicillin-clavulanic acid), fluoroquinolones (marbofloxacin), macrolides (tylosin, tulatromycin) and cephalosporins (cefotaxime, β-lactamic antibiotic, 3rd generation cephalosporin class) (Melamed et al., 2012) and was estimated by the Kirby Bauer diffusion method (Figure 2).

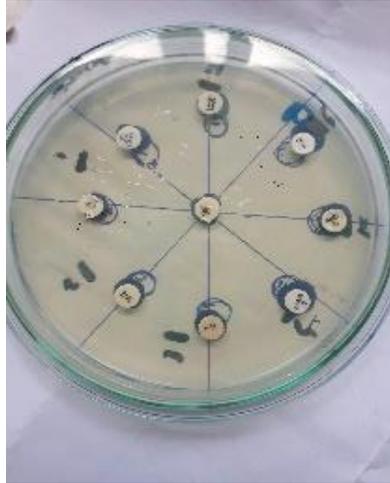


Figure 2. Kirby Bauer method

The MAR index was calculated, by using the formula $MAR = a/b$, where a represents the number of antibiotics to which the test strain depicted resistance and b represents the total number of antibiotics to which the test strain has been evaluated for susceptibility (Sandhu et al., 2016).

RESULTS AND DISCUSSION

After cultivation and colony isolation (pure colony culture were subjected to testing) (Figure 3 and Figure 4), strains from *Staphylococcus (sciuri and warnerii)* (Sun et al., 2015), *Shigella spp.*, *Kytococcus (sedentarius)*,



Figure 3. Aspect of bacterial strains isolated on simple broth



Figure 4. Colonies aspect on nutritive agar

Salmonella spp. and *Citrobacter (freundii)* genera and species were identified (i.e. Figure 5, Figure 6 and Figure 7), from the collected samples.

ERIC Web										Identification Report	
RapiD Staph Plu Laura S										Run Date: 6/18/2018	
Microcode: 073264										Facility: USAMV Cluj-Napoca	
										Reference No:	
System Tests											
-ADH	00%	+SUC	98%	+aGLU	56%	-GUR	16%	-PYR	03%	-LEU	21%
-ODC	00%	+MANO	93%	+BGLU	99%	+NAGA	98%	+ARG	00%	-LGLY	03%
-LIP	03%	+PO4	95%	-ONPG	00%	-URE	02%	+ALA	04%	+NIT	98%
QUESTIONABLE MICROCODE - Unreliable Probabilities											
Choice	Probability			Bioscore			Contraindications				
S. sciuri	> 99.9%			1/90814			ARG [0] ALA [4]				

Figure 5. Identification report of a *Staphylococcus sciuri* colony

ERIC Web		Identification Report	
RapID Staph Plu	S9 Laura	Run Date:	6/18/2018
Microcode:	432450	Facility:	USAMV Cluj-Napoca
Reference No:			
System Tests	-ADH 57% +SUC 81% -aGLU 92% -GUR 74% +PYR 24% -LEU 11%		
	-ODC 03% +MANO 07% +BGLU 89% -NAGA 01% -ARG 08% -LGLY 03%		
	+LIP 87% -PO4 04% -ONPG 03% +URE 84% +ALA 29% -NIT 33%		
QUESTIONABLE MICROCODE - Unreliable Probabilities			
Choice	Probability	Bioscore	Contraindications
S. warneri	> 99.9%	1/91540	MANO[7] aGLU[92] PYR [24]

Figure 6. Identification report of a *Staphylococcus warneri* colony

ERIC Web		Identification Report	
RapID Staph Plu	Laura s4	Run Date:	6/18/2018
Microcode:	100063	Facility:	USAMV Cluj-Napoca
Reference No:			
System Tests	+ADH 14% -SUC 00% -aGLU 99% -GUR 00% -PYR 03% +LEU 80%		
	-ODC 00% -MANO 00% -BGLU 00% -NAGA 00% +ARG 89% +LGLY 98%		
	-LIP 03% -PO4 51% -ONPG 00% -URE 00% +ALA 91% -NIT 04%		
Presumptive <i>K. sedintarius</i>			
Choice	Probability	Bioscore	Contraindications
K. sedintarius	> 99.9%	1/2562	ADH [14] aGLU[99]

Figure 7. Identification report of a *Kytococcus sedintarius* colony

Staphylococcus sciuri is a coagulase-negative, novobiocin-resistant, oxidase-positive staphylococcal strain, commonly present on animals' skin and mucosal surface (Dakic et al., 2005). Despite being a commensal bacterium, *S. sciuri* has been responsible for infections, both in animals and humans as a highly pathogenic agent, having a major role as gene resistance carrier/reservoir (Nemeghaire et al., 2014).

Staphylococcus warneri is a catalase-positive, oxidase-negative, and coagulase-negative bacteria, commensal, found in cutaneous microflora, both in animals and humans. It's an opportunistic pathogen, causing pathologies in immunocompromised subjects such as abortion and mastitis in cattle, urinary tract infection (UTIs), meningitis, endocarditis in dogs, etc. Its identification is usually associated to bovine presence in the testing area (Kloos et al., 1975; Barigye et al., 2007).

Shigella spp. and *Salmonella spp.* discovery were associated to poor hygiene conditions, common in low input small farms and pigs' habit to bath in feces and mud. Both bacteria species are responsible for infections of the digestive or respiratory tract or systemic infections. Their importance resides in their zoonotic potential (Harris, 2013). Finding these strains indicated the cohabitation of multiple species in the same area and the possibility of pathogen passage either intra- or interspecifically. Furthermore, the assumption could be made that these strains could have been transferred from animals to humans or the other way around, due to close relationships between these categories on low input, small swine farms.

Antimicrobial resistance test (Table 1) revealed that *S. warnerii*, an opportunistic pathogen, was the most resistant strain; nevertheless, the MAR index was high (0.33) in 50% of the strains. The most efficient antibiotic proved to be cefotaxime, because it is rarely used, since is quite costly and sometimes the owners cannot afford. The least efficient was oxitetracycline, most probably due to its large usage in veterinary therapies given its inexpensiveness.

The study also showed that multiple antibiotic classes can lead to induction of resistant colonies (RC), leading to the conclusion that 6 out of 9 antibiotics tested should be excluded from any therapeutic protocol in extensively raised swine.

Table 1. MAR index and inhibition diameter, of the tested antibiotics

Antibiotic Strain	CN mm	TUL mm	CTX mm	DO mm	S mm	AM C mm	MAR mm	T mm	TY mm	MAR Index
<i>Shigella</i>	18	24	21	20	19	R	24	R	R	0.33
<i>K. sedintarius</i>	22	29	21	23	22	8	21	R	R	0.22
<i>S. sciuri</i>	17 + RC	16	18	31	16 + RC	24	19	28	18	0.22
<i>S. warneri</i>	18 + RC	10 + RC	17	16	21	19	21	R	17	0.33
	2CR	1CR	sens	sens	1 RC	1R	sens	3R	2R	

Notes: CN – gentamicin, TUL – tulathromycin, CTX – cefotaxime, DO – doxycycline, S – streptomycin, AMC – amoxicillin clavulanic acid, MAR – marbofloxacin, T- oxytetracycline, TY – tylosin, R-resistant, RC- resistant colonies, sens-sensitive

Antibiotic resistance of bacterial organisms, is a persistent, global health threat, due to its association with high mortality and morbidity that affects both human and animals. The finding of Gram-positive and -negative bacteria, that possess multidrug resistance, are a challenge in therapy, due to their ability to be unresponsive to conventional antibiotics. Currently, worldwide is a shortage of effective antibacterial therapies and only a few new antibiotics, which lead to unsuccessful infection control (Frieri et al., 2016).

The finding of resistant strain of *S. warnerii* on swine skin, usually found as a skin commensal of humans (Kanuparty et al., 2020), indicates the close interaction between humans and animals, the possibility of cross-species transmission and an increased opportunity for these bacteria to develop antibiotic resistance (Tang et al., 2017).

CONCLUSIONS

The results indicate the presence of antibiotic resistant ubiquitous and pathogenic strains within the tested subjects' cutaneous microbiome which need caution, since they could express pathogenicity under appropriate conditions, which free-range system could provide (continuous environment changes; biased therapeutic protocols, non-discriminatory antibiotic therapy, etc.). It would be useful for the practitioners who support this kind of small farms or back-yard raising farms to constantly monitor the antimicrobial resistance in their area in close connection to antimicrobial therapy provided to all animal species and tailor the therapeutic protocols implemented based on the obtained MAR results.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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