Antibiotic Resistance Profile of *Escherichia coli* and *Klebsiella spp* Isolated from Post-weaned Piglet Faeces under Penicillin-streptomycin Combination Therapy (Penstrep®) in Abidjan, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author ANTK designed the work, wrote protocol, carried out the experiments and wrote the first draft of manuscript. Authors NKN, BKG and MBO analyzed data obtained, authors ANTK and FKK carried out literature searches. Authors AD and NKG read and approved the final manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The current study was aimed at evaluating the antibiotic resistance profile of *Escherichia coli* and *Klebsiella spp* isolated from post-weaned piglets treated with penicillin-streptomycin combination therapy.

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Study Design: Bacteriological study.
Place and Duration of Study: Laboratory of the National Reference Center for antibiotics at Institut Pasteur Côte d'Ivoire, between March 2018 and June 2018.
Methodology: *Escherichia coli* and *Klebsiella* species were isolated from post weaned piglets stool on Mac Conkey medium added up separately with penicillin, streptomycin and combined penicillin-streptomycin and identified. Antibiotic susceptibility test was performed using disk diffusion method on Müller-Hinton agar.
Results: *Escherichia coli* and *Klebsiella* species resistance to penicillin, streptomycin and combined penicillin-streptomycin evaluated, respectively, reached 80.5% (Day 0) to 92.0% (Day 4); 17.0% (Day 0) to 39.9% (Day 4) and 31.3% (Day 0) to 70.9% (Day 4) for piglets treated with Penstrep®. In addition, antibiotic susceptibility test carried out for *Escherichia coli* and *Klebsiella spp* strains isolated from piglets treated with Penstrep® revealed resistance to amoxicillin for each bacterial species at a rate of 86.0% and 89.0%. Furthermore, control piglets showed lesser resistance to streptomycin (*E.coli* 31.0% and *Klebsiella spp* 38.0%) than those treated with the combination therapy Penstrep® (*E. coli* 73.0% and *Klebsiella spp* 48.0%). As for netilmicin, imipenem and colistin, no resistance was detected for treated piglets as well as untreated ones.
Conclusion: The combination therapy with Penstrep® has increased *Escherichia coli* and *Klebsiella* species resistance to antibiotics tested in the current study.

1. INTRODUCTION

Enterobacteria constitute a large family of Gram-negative bacteria responsible for various, diseases with different pathogenic mechanisms [1] and as a result, is of great importance in human infections. This importance can be explained by the variety of bacterial species which compose them as to their impact on population's health. The frequency and severity of the infections for which they are responsible (septicemia, Nosocomial infections etc) and their resistance to antibiotics translate difficulties of care. In fact, species from this bacterial family are extensively exposed to commonly used antibiotics [2,3]. Unfortunately, these microorganisms previously sensitive to them, have completely become resistant. This results from several complex interactions between bacteria and the environment, especially because antimicrobials frequently used and misused in human and veterinary medicine [4]. For example, antimicrobials are used as food supplements and growth promoters [5]. In the swine industry, they are mostly used in post-weaning piglet's diarrhea prophylaxis, as well as growth promoters for extensive breeding [6]. The combination penicillin-streptomycin is one of the most used antibiotics on farms in Côte d'Ivoire particularly in swine breeding. This combination is used to prevent arthritis in piglets or for the control of respiratory and digestive infections that start in the post-weaning period [7]. This misuse of antibiotics in pig breeding exerts a selection pressure which favors the emergence and dissemination of multidrug resistant bacteria. To cope with the selection pressure exerted by antibiotics, bacteria exploit mechanisms that allow them to adapt to the hostile conditions of their environment [4,8]. In the digestive tract of animals, selection pressure could contribute to zoonotic resistant bacteria, which are pathogenic to humans [9,10]. Dissemination of antibiotic resistant bacteria in human population, pose a threat to public health. This study was conducted to determine antibiotic resistance profile of *Escherichia coli* and *Klebsiella spp* isolated from the faeces of post-weaned piglets treated with penicillin-streptomycin combination therapy.

2. MATERIALS AND METHODS

2.1 Sampling

Four post-weaned piglets were used for this study. Two of the piglets had never been treated with antibiotics (batch 1: control) and the other two piglets also having not been treated with antibiotics before the study, received the penicillin-streptomycin combination therapy Penstrep® (batch 2: treated).

The following break-down of the experimental period was adopted. The day prior to start experiment was set at Day 0, and the other days were named Day 1, 2, 3 and Day 4. Then, piglet faeces from batch 1 and batch 2 were collected

over 5 days period using labeled sterile pots, transported in icebox containing ice packs to the laboratory for analysis.

2.2 Isolation and Identification of *Escherichia coli* and *Klebsiella* species

Isolation of *Escherichia coli* and *Klebsiella* species was carried out in the Laboratory of the National Reference Center for antibiotics at Institut Pasteur Côte d'Ivoire.

Five gramme (5 g) of piglets faeces were homogenized in a 45 mL of peptone water, and 1/10 serial dilutions were made from stock solution. Then, 100 µL of these dilutions were streak-seeded on Mac conkey agar medium. For each sample, two Petri dishes were seeded on Mac Conkey medium previously impregnated with 17 µg/mL of penicillin and 17 µg/mL of streptomycin (MC+PEN, MC+STREP, MC+PEN+STREP).

Powdered antibiotics inoculated on to the Mac Conkey medium were set according to the minimum inhibition concentration standards, according to the standard value of the Committee of Veterinarians of the French Society of Microbiology [10]. In addition, *Escherichia coli* and *Klebsiella* species that grow on that Mac Conkey medium were qualified as resistant. The bacteria on different media MC, MC+PEN, MC+STREP, MC+PEN+STREP) have allowed to alculate the percentage of resistant bacteria according to the formula below.

$$\% R = \frac{n_{MC+ATB}}{n_{MC}} \times 100$$

% R : percentage of enterobacteria resistant to an antibiotic

n_{MC+ATB} : number of colonies counted on medium made up of Mac Conkey + antibiotic

n_{MC} : number of colonies counted on Mac Conkey medium

Bacteria colonies isolated on each Petri dish MC+PEN, MC+STREP and MC+PEN+STREP for control and treated piglets with Penstrep® for 5 days (Day 0, Day 1, Day 2, Day 3 and Day 4) were identified by using biochemical tests (oxidase, catalase and the media of the reduced

rack of Leminor). A total of 180 strains were identified in this study.

2.3 Antibiotic Susceptibility Test of *Escherichia coli* and *Klebsiella* species

The antibiotic susceptibility testing of the previously isolated germs (*Escherichia coli* and *Klebsiella*) was carried out by diffusion disk method on Muller Hinton Agar according to the standard value of the Committee of Veterinarians of the French Society of Microbiology [11]. Inhibition zone diameters were read with a vernier caliper and interpreted as sensitive, intermediate or resistant, in line with standard critical diameters for enterobacteria. Twenty-four (24) antibiotic disks were used in the current study as follows: amoxicillin (20 µg), amoxicillin / clavulanic acid (20 µg); piperacillin (30 µg); piperacillin / tazobactam (30 µg); ticarcillin (75 µg); ticarcillin / clavulanic acid (75 µg); streptomycin (10 µg); cefepime (30 µg); cefotaxime (5 µg); ceftazidime (10 µg); cefoxitine (30 µg); imipenem (10 µg); aztreonam (30 µg); ciprofloxacin (5 µg); nalidixic acid (30 µg); amikacin (30 µg); gentamicin (10 µg); netilmicin (10 µg); tigecycline (15 µg); tetracycline (30 µg); chloramphenicol (30 µg), fosfomycin (20 µg); trimethoprim-sulfamethoxazole (1.25-23.75 µg); colistin (50 µg).

3. RESULTS

3.1 Prevalence of Bacteria Resistance in Control and Penstrep®-Treated Piglets

Penicillin, streptomycin and penicillin-streptomycin resistance rates in control and Penstrep® treated piglets are as shown in Fig. 1 and Fig. 2. These rates are higher for penicillin and relatively low for streptomycin and the penicillin – streptomycin combination in both batches of piglets. However, the resistance trend is higher in bacteria isolated in treated piglets.

3.2 Identification of Resistant Bacteria

Identification of penicillin and streptomycin-resistant bacteria in the control and Penstrep® piglets groups revealed a higher proportion of *Escherichia coli* in both control (75.5%) and treated samples (77.7%) followed by *Klebsiella spp* with 21% and 14.4% in control and treated piglets (Fig. 3).

3.3 Antibiotic Susceptibility Test

Fig. 4, Fig.5, Fig.6 and Fig.7 show the antibiotics susceptibility test results of *Escherichia coli* strains isolated in control and treated piglets. Resistance rates for all antibiotics tested on *Escherichia coli* isolated from control piglets are below 50.0% both on D0 and D4. The highest resistance was observed with tetracycline with 48.0% (D0) and 41.0% (D4). These strains showed good sensitivity to aminoglycosides, carbapenems, fluoroquinolones and polymixins. *Escherichia coli* isolated in penicillin-streptomycin treated piglets presented good sensitivities before antibiotic treatment. At the end of

treatment on day D4, an increase in resistance was observed generally for all antibiotics except for imipenem, amikacin, netilmicin and colistin which remained very active on these strains.

Fig.7, Fig.8, Fig.9, Fig.10 and Fig.7 show these sensitivity results for *Klebsiella spp*. These strains exhibited relatively high resistance in control piglets with a maximum for amoxicillin (57.0% D0 and 78.0% D4) and tetracycline (57.0% D0 and 65.0% D4). No resistance was observed in *Klebsiella spp* with fosfomicin, imipenem, amikacin, gentamycin, netilmicin, tigecycline and colistin both on D0
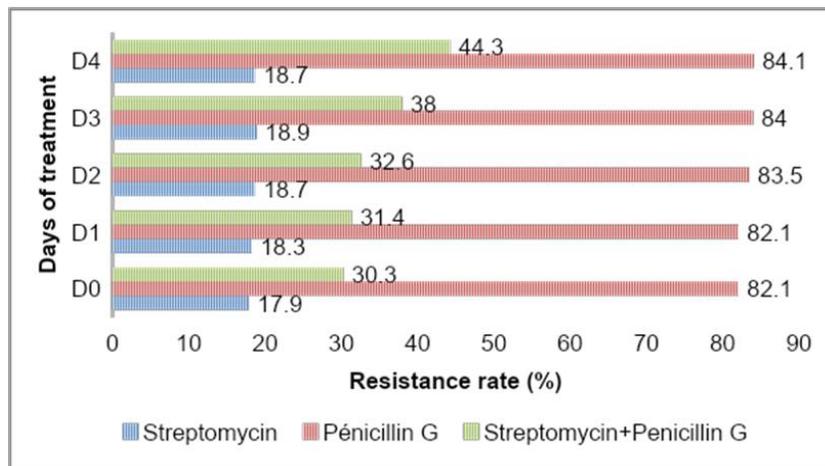

Fig. 1. Penicillin, streptomycin and penicillin-streptomycin combination therapy and bacterial resistance rates in control piglets
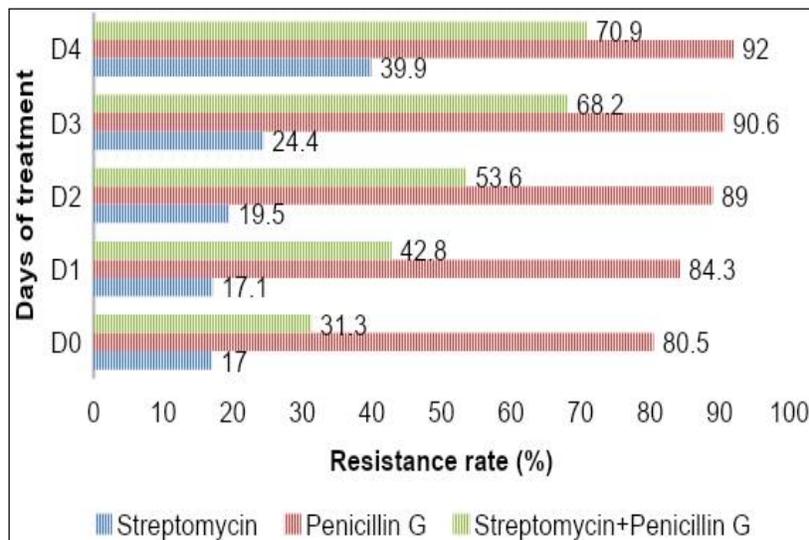

Fig. 2. Penicillin, streptomycin and penicillin-streptomycin combination therapy and bacterial resistance rates in treated piglets

and D4 in control piglets. From these figures, an increase antibiotic resistance for *Klebsiella spp* was observed in piglets treated with penicillin-streptomycine combination therapy without any resistance to fosfomycin, imipenem, amikacin, netilmicin, tigecycline and colistin on days D0 and D4.

4. DISCUSSION

Escherichia coli and Klebsiella species isolated from control and piglets treated with combination therapy (Penstrep) exhibited high resistance to penicillin. A resistance rate of 82.1% and 80.5% was recorded for the control and piglets treated with combination therapy respectively. These resistance rates increased slightly from 82.1% on day 1 to 84.1% on day D4 in the case of control piglets. To illustrate, piglet's microbiota shows intrinsic resistance genes, even in isolates that have never been exposed to antibiotics [12].Furthermore, resistance rate to Penicillin for Penstrep[®] treated piglets increased significantly from 84.3% on day 1 to 92.0% on day 4. This
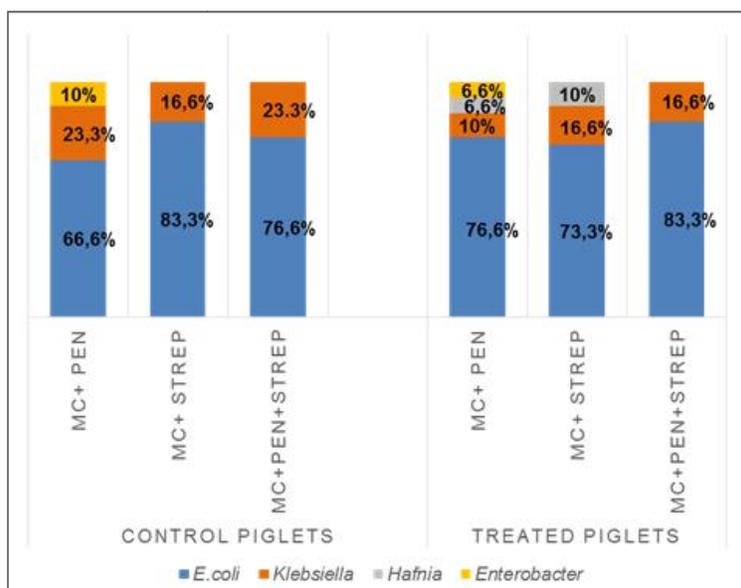

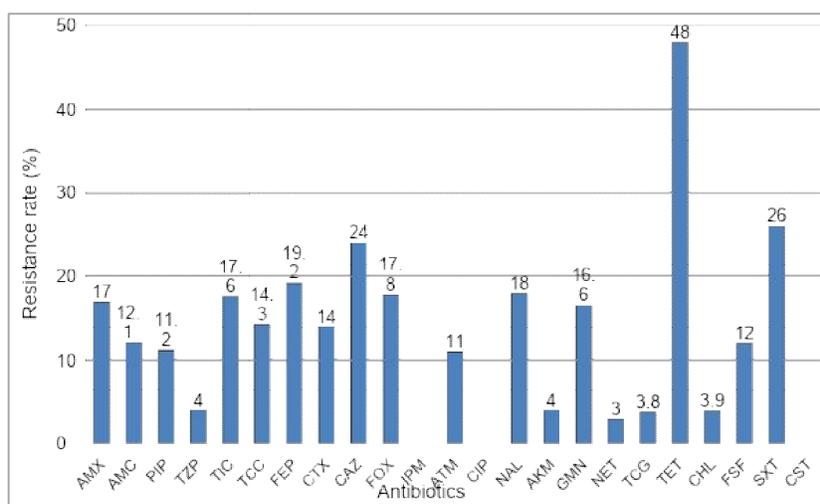**Fig. 3. Bacteria strains resistance rates to penicillin, streptomycin and penicillin-streptomycine**

Fig. 4. Antibiotic susceptibility profile of *Escherichia coli* isolated from control piglets on D0
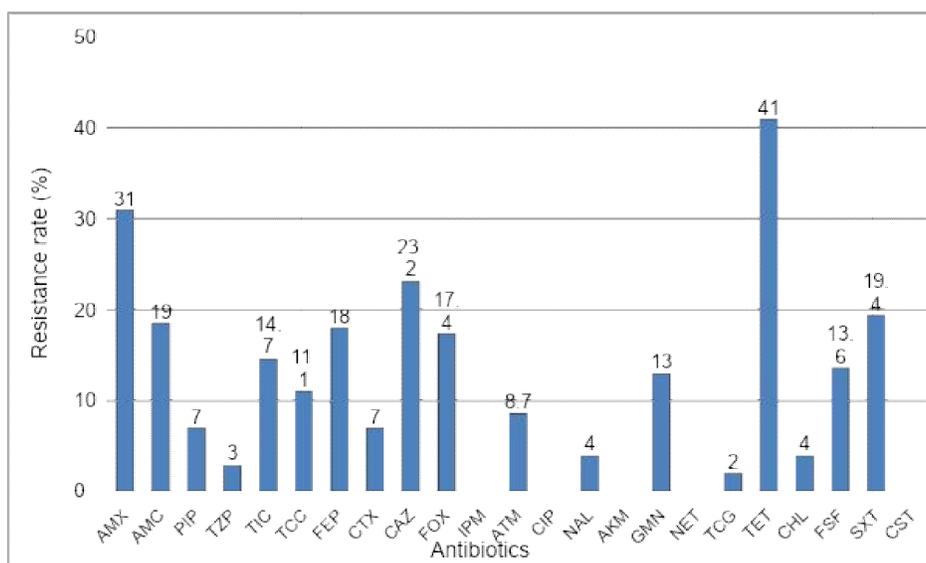
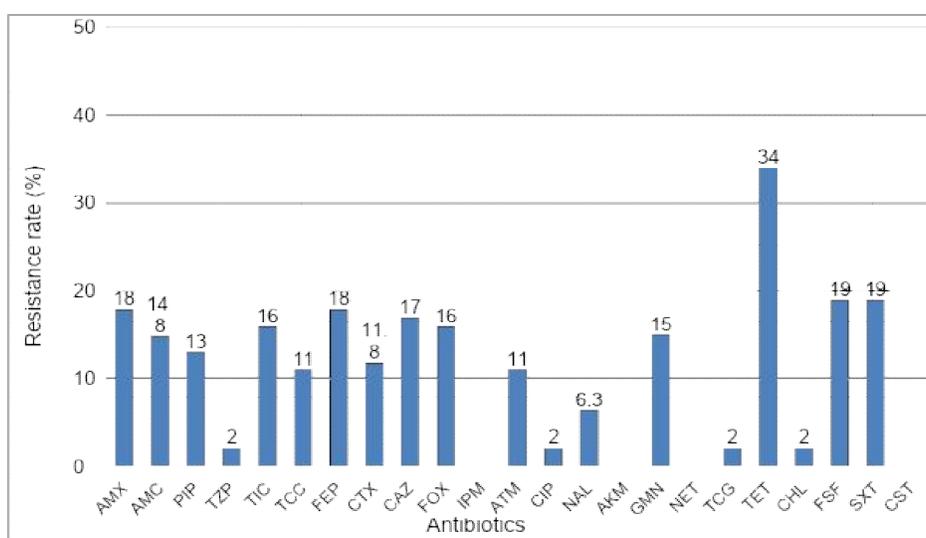**Fig. 5. Antibiotic susceptibility profile of _Escherichia coli_ isolated from control piglets on D4**

Fig. 6. Antibiotic susceptibility profile of _Escherichia coli_ isolated from treated piglets on D0

trend could be explained by exposure of isolates to penicillin over the four days treatment period, as well as by natural resistance of Gram-negative bacteria to penicillin G. Indeed, enterobacteria are part of species that present intrinsic resistance to penicillin G [13,14,15]. Since Penstrep® is a combination of penicillin G and streptomycin, the higher resistance observed in control and treated piglet faeces before treatment started, could be explained evidently. However, resistance levels are relatively lower for streptomycin during treatment from 17.9% (D0) to 18.7% (D4) for control piglets and from 17.0% (D0) to 39.9% (D4) for treated piglets. It should also be noted that the resistance rates of these enterobacteria increases more rapidly in treated piglets exposed to streptomycin as compared to control piglets. The rapid development of penicillin and streptomycin resistant enterobacteria in treated piglets may be explained by the previous exposure of these bacteria to the mentioned antibiotics. Steady exposure to an antibiotic favors survival of resistant bacterial strains present in a population [16,17]. In addition, streptomycin has broad-spectrum bactericidal activity on specific gram-negative bacilli, gram-positive cocci or mycobacteria [18]. Penicillin also demonstrates
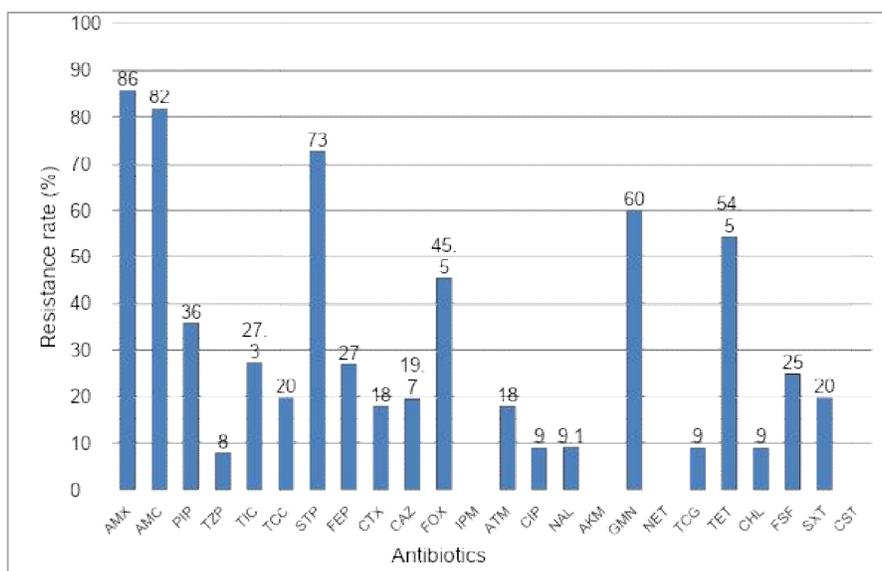
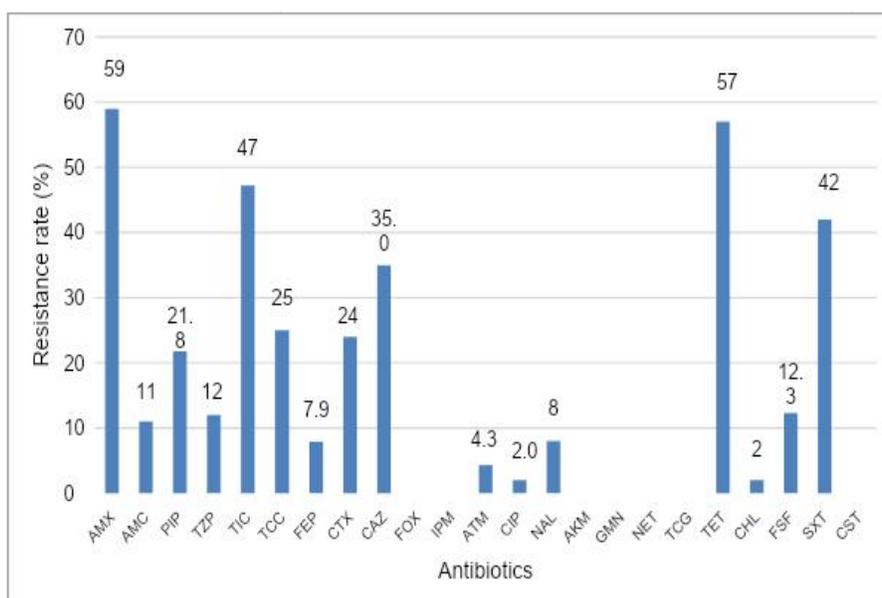**Fig. 7. Antibiotic susceptibility profile of *Escherichia coli* isolated from treated piglets on D4**

Fig. 8. Antibiotic susceptibility profile of *Klebsiella spp* isolated from control piglets on D0

biological effect on Gram-positive and some specific Gram- negative bacteria. Combination therapy with penicillin G and streptomycin would extend activity spectrum in case of mixed microbial infections. Another investigation showed synergistic efficacy of penicillin-streptomycine combination in comparison with two antibiotics used alone [19]. However, the use of this combination therapy has resulted in increased resistance of enterobacteria. The resistance of enterobacteria to penicilline-streptomycine combination therapy (31.3% on day 0 to 70.9% on day 4) was higher than the resistance to streptomycin taken alone but low compared to resistance to penicillin alone. This emergence of resistance to these antibiotics during periods of antibiotic treatment in animals could be explained by the presence of pre-existing resistant organisms before treatment or of resistant gene transfer [20,21] Antibiotic susceptibility profile of *Escherichia coli* and *Klebsiella spp* isolated from control piglets

exhibited low resistance to separate antibiotics as compared to Penstrep® treated piglets. Therefore, it is suspected that antibiotic resistance was increased by Penstrep® treatment. As for veterinary medicine in general, and swine farming in particular, antibiotic used play a crucial role in the development of antibiotic resistance in normal microbiota.

This is ascertained by [22] who found that 26.0% of coliforms in piglets were resistant to tetracycline in a herd not exposed to antibiotics and 76.0% in another herd treated with antibiotics. Nevertheless, antibiotic susceptibility tests conducted in this study revealed activity of imipenem, netilmicin and colistin on *Escherichia coli* and *Klebsiella spp* in control and treated piglets. Similar results were observed with imipenem [23,24,25]. Finally, antibiotic susceptibility tests on *Klebsiella spp* showed higher resistance rate to amoxicillin (78.0%) in control and 89.0% in treated piglets. While
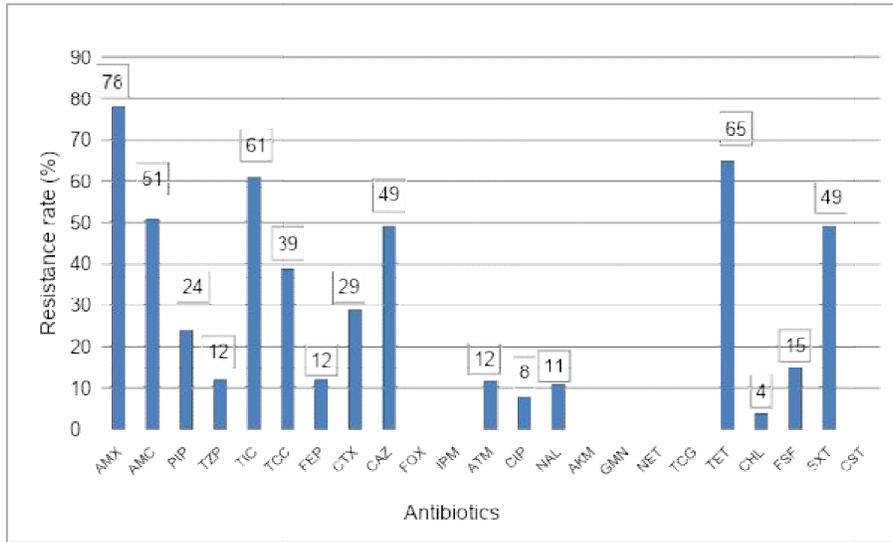

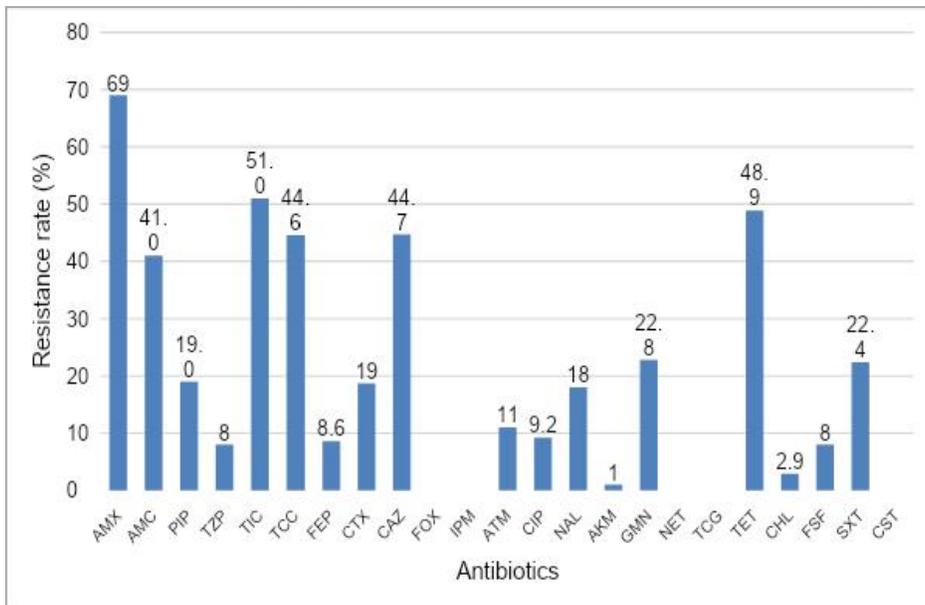**Fig. 9. Antibiotic susceptibility profile of *Klebsiella spp* isolated from control piglets on D4**

Fig. 10. Antibiotic susceptibility profile of *Klebsiella spp* isolated from treated piglets on D0
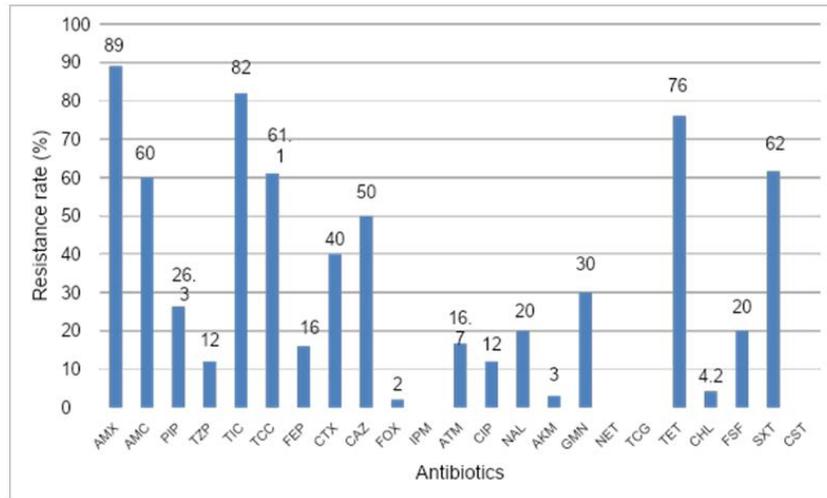
Fig. 11. Antibiotic susceptibility profile of *Klebsiella spp* isolated from treated piglets on D4

Escherichia coli percentage resistance were 31.0% and 86.0%, respectively for control and Penstrep® treated piglets. These relatively high percentages in treated piglets could be due to exposure of these piglets to penicillin when treated with Penstrep®. The rapid increase in the resistance of *Escherichia coli* and *Klebsiella spp* to antibiotics tested in treated piglets is due to their exposure to penicillin-streptomycin during treatment. The use of an antibiotic contributes to gradually enrich the bacterial population of multi-resistant strains and whatever the antibiotic used, it contributes globally to the selection of resistant strains [21]. Strains resistance levels are generally very high compared to antibiotics widely used in the swine industry, such as amoxicillin or tetracycline [26]. These resistant bacteria of porcine origin, can be directly transmitted to human (by food or by direct contact with the pig) and entail either a toxi-infection, or a propagation of resistance genes to commensal and infectious bacteria of human origin [9]. It has been gradually shown that human and animal bacteria can transfer their resistance when they come into contact. Animal antimicrobial resistance can therefore feed human antimicrobial resistance. The resistance of *Escherichia coli* and *Klebsiella spp* (of porcine origin) to antibiotics widely used in human medicine such as amoxicillin, ciprofloxacin and gentamycin could then lead to public health problems.

5. CONCLUSION

This study shows that the use of penicillin-streptomycin combination in piglet treatment increases both prevalence of resistance for *Escherichia coli* and *Klebsiella spp* to penicillin and streptomycin with prevalence of resistance for these isolated bacterial to other antibiotics. Yet, some antibiotics, namely imipenem, netilmicin and colistin were effective in control and treated piglets for both *Escherichia coli* and *Klebsiella spp*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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