

Prevalence and antimicrobial resistance of bacteria isolated from Beni-Guil lamb carcasses in eastern Morocco

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Abstract

This study aims to assess the microbial quality and antimicrobial resistance of bacteria isolated from Beni-Guil lamb carcasses. 103 samples were used for the study. The mean values obtained for microbial quality were 4.65, 3.07, 1.26, 0.99 and 0.69 (log₁₀ CFU/cm²), respectively for total aerobic Flora counts, *Enterobacteriaceae*, fecal coliform, *Escherichia coli* and *Staphylococcus aureus*. 70% of tested *Escherichia coli* strains were resistant to gentamicin and ciprofloxacin, as for *Staphylococcus aureus*, 100% of strains tested were resistant to ciprofloxacin and sensitive to erythromycin. Regarding gentamicin and benzylpenicillin, 70 % and 60% of *Staphylococcus aureus* strains were resistant, respectively.

Keywords: Beni-Guil sheep, Carcass, Microbial quality, Antimicrobial resistance

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Introduction

In Morocco, sheep farming is an important component of the rural economy, particularly in arid, semi-arid and mountainous areas of the country (Boujenane, 2005; Berrighi et al., 2017). It occupies a vital and active role in the animal production field and it's considered as the second supplier of red meat after cattle's (Chemonics International, 2006). In Morocco, the total livestock exceeds 29 million head, with sheep counting for 20 million head (HCP, 2017). Animal products, mainly meat and its products are responsible

for an important number of foodborne diseases due to ingestion of pathogenic bacteria such as *Staphylococcus aureus* and *Enterobacteria*, which are considered among the most significant causes of food poisoning in the world. In Morocco, meat and its products are the most incriminated foodstuff with a prevalence of 25.8% (CAPM, 2018). Due to its origins, meat is the main agent of transmission of pathogenic and resistant bacteria and can be contaminated upstream or downstream of the production chain. However, unhygienic handling during slaughter and preparation of carcasses leads to important superficial



contamination which can affect the consumer's health and the meat quality (organoleptic deterioration) (Dennai et al., 2000). In Morocco, the slaughterhouse appears to be one of the major critical points in terms of meat hygiene. In fact, it is estimated, that 80 to 90% of the contaminations of meat by microflora arriving at consumers results from the slaughterhouse (Jouve, 1990).

Antimicrobial resistance is a major and global public threat to both human and animal health. This phenomenon has become a globally widespread problem. The monitoring of this problem plays an important and key role in sustainable development (Rather et al., 2017). It can infect anyone, anywhere. There are numerous mechanisms by which microorganisms acquire resistance to drugs. The latter can be encoded on the chromosome or on plasmid as a transmissible element. Lamb meat of the Beni-Guil breed labelled protected geographical indication is one of the main meats produced among Morocco meats. It is very appreciated by the consumer because of its taste and its nutritional quality (Belhaj et al., 2018). Although the meat microbiological quality has been the subject of numerous studies, to our knowledge, there are no studies, on one hand, on surface contamination of sheep carcasses using the non-destructive method and, on the other hand, on the antimicrobial resistance of bacteria isolated from the surface of sheep carcasses. Considering the socioeconomic importance of this breed, and given the large consumption of its meat in the eastern regions as well as others regions of the country, it is important for us to study the microbiological risks associated to this foodstuff. The study revolves around the assessment of the microbial quality and antibiotic-resistance of bacteria isolated from the carcasses of Beni-Guil lamb.

Material and Methods

Carcass sampling site

In this study, 103 samples were tested. The sampling was carried out at two slaughterhouses in the eastern region of Morocco during the year 2018 between March and May. The analyses were conducted in the Laboratory of Analysis and Research of the National Office for Food Safety which works according to quality assurance conditions (ISO 17025).

Sampling and transport of samples

The sampling methods, the selection of the collection sites and the rules for storage and transportation of

samples have been made in accordance with ISO 17604. The sampling was carried out weekly. In the same slaughtering session, 5 carcasses were sampled before chilling. As for aerobic colony counts and *Enterobacteria* daily assessment, the log mean of 5 carcasses (4 areas of 100 cm² on each carcass) was required. However, for *Salmonella*, 50 carcasses were needed for criteria assessment. The samples were collected aseptically, maintained at 6°C in a thermoelectric cooler with chiller ice packs, and transported to the laboratory.

Microbiological test

The microbiological tests were carried out on the same day. Among the isolated microorganisms, 20 bacteria (10 *E. coli* and 10 *S. aureus*) were selected for the antibiotic resistance test.

All microbiological tests were carried out according to ISO standard methods: Enumeration of the Total Plate Count (TPC) in accordance with ISO 4833-1 (ISO, 2013), enumeration *Enterobacteriaceae* in accordance with ISO 21528-2 (ISO, 2017) and fecal coliforms using V 08-060 (ISO, 2009). The enumeration of *S. aureus* was carried out in accordance with ISO 6888-2 (ISO, 1999). The detections of *Salmonella* spp and *Listeria monocytogenes* were performed according to ISO 6579 (ISO, 2002) and ISO 11290-1 (ISO, 2004) respectively. Sample preparation and decimal dilutions were done according to the ISO 6887-2 (ISO, 2010) standard method.

Antibiotic resistance tests

The study of antibiotic resistance was carried out in 3 essential steps according to the standards and recommendations of the French Society of microbiology's antibiogram committee "Comité de l'Antibiogramme de la Société Française de Microbiologie" (SFM, 2018):

- The culture of the purified bacterial strains (originally found in sheep carcasses) in Mueller-Hinton broth which is used as a non-selective medium to grow a very large number of bacteria of various origins by transferring them in the broth by using sterile inoculating loops and incubating at 37°C for 24 hours.
- Swab inoculation of the bacterial strains is grown in Mueller-Hinton broth on Mueller-Hinton agar
- Depositing of antibiotic discs, then incubation of agar plates at 37°C for 24 hours.

The isolated bacteria were tested against various families of antimicrobials, the following antibiotics were used: Amoxicillin (25µg), nitrofurantoin



(300µg), Co-trimoxazole (1.25/3.75µg), Erythromycin (15µg), Tetracycline (30µg), Thiamphenicol (30µg), Benzylpenicillin (6µg). For each antibiotic, the diameter of the inhibition zone was measured and the bacteria’s clinical category in regard to each tested antibiotic (susceptible, intermediate, resistant) was determined (SFM, 2018).

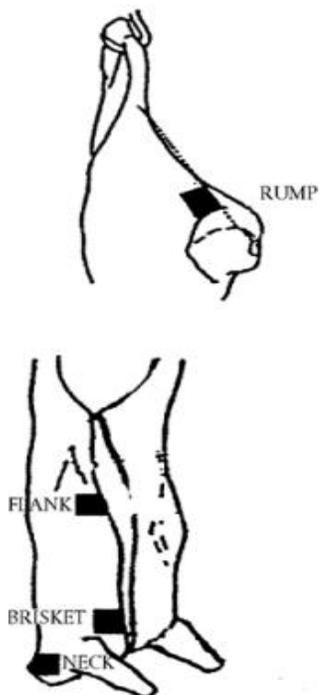


Figure-1: ISO 17604 Suggested sampling sites for ruminants(ISO, 2015)

Results and Discussion

Microbial quality of Beni-Guil sheep carcasses

This research made it possible to evaluate the surface contamination of sheep carcasses of the Beni-Guil breed by counting indicators of hygienic quality (total aerobic mesophilic flora and *enterobacteria*) and searching for indicators of the sanitary quality of the carcass (*Salmonella* spp, *listeria monocytogene*, and *Escherichia coli* O157: H7).At the same time, a bacteriological investigation and evaluation of the materials, premises, equipment, staff members and working/operating conditions is was carried out. The results of the bacteriological quality of the analyzed samples are presented in Table 2.The microbial quality of carcasses examined translates the hygienic measures adopted during the slaughter chain and post-processing of such perishable foodstuff. The

results show that 20 bacteria were isolated (10 *E. coli* and 10 *S. aureus*), from 103 samples. The enumeration of the hygienic indicator criteria (hygienic quality) shows that the average value of germs is 4.65 log₁₀CFU/cm²for ACC which constitutes the predominant flora, followed by *Enterobacteriaceae* with an average of 3.07 log₁₀ CFU/cm², then *E. coli* and *S. aureus* with averages of 0.99 and 0.69 log₁₀ CFU/cm² respectively. The results of the microbiological analysis showed that 24.27% of samples are of satisfactory quality (below m), 29.12% are of acceptable quality (between m and M) and 46.61% are of unsatisfactory quality according to the standards (Table 1). Our results of TCC are lower than those reported by Mocho (2005) for sheep carcasses in France. However, they are higher than those reported by Okraszewska-Lasica et al. (2012) and Phillips et al. (2013) for sheep carcass in Ireland and Australia respectively.

Table-1. Acceptability threshold required in carcasses samples according to European standards.

	ACClog ₁₀ CFU/cm ²	<i>Enterobacteri</i> <i>arial</i> log ₁₀ CFU / cm ²	<i>Salmonella</i> sppin 10 g	<i>Listeria monocytogenes</i> in 10 g	<i>E. coli</i> O157:H7
m	3.5	1.5	Abs	Abs	Abs
M	5	2.5	Abs	Abs	Abs

ACC: Aerobic Colony Counts; *E. coli*:*Escherichia coli*; m: fixed criterion; M: Maximum threshold or acceptability limit.

Table-2. Charge (log₁₀CFU/ cm²) of microorganisms in Beni-Guil carcasses samples collected in eastern Morocco

	Mean(log ₁₀ CFU/c m ²) ± Standard deviation	Criteria (m) (log ₁₀ CFU/c m ²)
ACC	4.65±0.57	3.5
<i>Enterobacteriaceae</i>	3.07±0.71	1.5
Fecal coliforms	1.26±0.57	1.5
<i>E. coli</i>	0.99±0.65	---
<i>S. Aureus</i>	0.69±0.64	---
<i>Salmonella</i> spp.	Abs	Abs
<i>Listeria monocytogenes</i>	Abs	Abs
<i>E. coli</i> O157:H7	Abs	Abs

ACC: Aerobic Colony Counts; *E. coli*: *Escherichia coli*; *S. Aureus*: *Staphylococcus aureus*

Concerning *Enterobacteriaceae*, the obtained average is higher than the one found by Okraszewska-Lasica et al. (2012) and Djenidi (2016) for sheep carcasses in Ireland and Algeria respectively. Regarding the fecal coliforms, the recorded values are lower than those reported by Goudiaby (2005), Mocho (2005) and Nouichi and Hamdi (2009) in Senegal, France and Algeria respectively. Meanwhile, they are higher than those reported by Okraszewska-Lasica et al. (2012) and by Omer et al. (2015). The obtained average value of *Escherichia coli* is higher than that reported by Phillips et al. (2013) in Australia. Finally, concerning the research of photogenic bacteria (safety criteria), our results show the absence of *Salmonella* spp, *Listeria monocytogenes* and *E. coli* O157:H7 in all analyzed samples. In comparison with the studies mentioned above, the Béni-Guil carcasses have a satisfactory sanitary quality, since Phillips et al. (2001), Nouichi and Hamdi (2009) and Hanlon et al. (2018) have indicated the presence of *Salmonella* spp and *E. coli* O157:H7 in small ruminant carcasses in Australia, Algeria and Bahamas respectively.

Regarding coagulase-positive *Staphylococcus*, our results are higher than those reported in sheep carcass in Australia by Phillips et al. (2013), however, they are lower than those reported by Djenidi (2016) in Algeria. Overall, the tested carcasses have a relatively high microbiological contamination indicating inadequate hygiene measures adopted during the preparation of this product. A similar result was reported by El Kadmiri et al. (2016) in Hay Hassani district-Casablanca (Morocco). This result can be explained as the cross-contamination of the carcass surface by finished and unfinished products which might have occurred due to contact with the skin or intestinal content during the skinning and evisceration step and by handlers from their hands in case of *Staphylococcus aureus*. Generally, the high-level contamination by superficial bacteria is an indicator of a lack of compliance with good manufacturing practices (GMP) and good slaughter practices (broken cold chain, improper cooling, hygiene and sanitation, etc.). So, the recorded difference between our study and the studies mentioned above can be clarified by GMP compliance and by the treatment of air and carcass surface by organic acids which decrease the surface contamination.

The results of the microbiological analyses of Beni-Guil sheep carcasses can be explained by the results obtained from the evaluation of the bacteriological quality of the equipment and the environment of the

slaughterhouse where the study took place. This evaluation made it possible to detect major hygienic defects (data is not shown).

In fact, hygienic defects were detected during the preparation of the animals (or first transformation). These are mainly issues with the hygiene of materials, premises, equipment, staff and working or operating conditions. The fundamentals of the go-forward principle and the separation of dirty and clean areas were not respected. The doors and openings remained open which facilitated the free circulation of wind, insects including flies and staff members from one sector to another, the latter can be potential vectors of microorganisms. Bleeding and skinning in the horizontal position, in other words in the lying down position, remains a traditional and ancient habit in the slaughtering process. This practice is the main source of carcass contamination. It is for this reason that the skin is considered a major critical point in terms of meat hygiene. To this, we must add, on one hand, the insufficient cleanliness of the animals before slaughter, illustrating a significant microbial load of the skin. On the other hand, there's also the bleeding carried out on the ground associated with the washing of the sheep. These practices increase the initial microbial load of the skin. Indeed, Fournaud et al. (1978) have obtained values five or even ten times greater than the initial microbial load of the skin, after a washing that was not followed by drying. The skin thus constitutes an important source of contamination of the carcasses. This skin contamination occurs mainly during dressing. It is now well established that the skin and the digestive tract are the 2 main sources of carcass contamination (Cartier and Moevi, 2007). The materials made with oxidizable iron were in a very advanced state of rusting. Their cleanliness and maintenance was insufficient. Thus, it was found that the wastewater drainage system was defective, particularly in the bleeding area due to the absence of gut protection grids. The rusty materials, unsatisfactory physical and hygienic maintenance of the equipment promote bacterial contamination of carcasses. Insufficient body and clothing hygiene make the staff the main vector of germs and contribute to the increase of the microbial load. Thus, the presence of foreign people (The meat wholesalers and their workers ...) during the slaughtering process constitutes a problem in terms of hygiene in the Oujda slaughterhouse. It is also noted that animal stalling and antemortem inspection are not systematic. This presents enormous risks, especially since this



operation has an important objective concerning bacteremia (reduction of the microbial load of the digestive tract) and more or less the correction of defects due to fatigue and stress (biochemical quality of the meat), as well as the elimination of sick animals. Thus, for the improvement of the hygienic quality and a better control of the bacteriological quality of sheep carcasses of the Beni-Guil breed, the following recommendations are proposed to improve the general hygiene of the slaughter line:

Improvement of the hygiene of materials and premises (doors, openings, floor and equipment): use of stainless equipment, installation of cleaning and disinfection stations for staff, the implementation of a cleaning and disinfection system to properly maintain the premises and the equipment and the installation of doors and screens on all the openings on the walls and the floor, to prevent the penetration of unwanted animals: dogs, cats, insects.

Improvement of staff hygiene, providing the workers with work uniforms, monitoring of clothing hygiene, the prohibition of the entry of foreign people and prohibition of changing workstations.

The cleanliness of incoming animals: it is, therefore, necessary to reduce the number of very dirty animals arriving at the slaughterhouse. For this we can consider charging meat wholesalers for soiled animals. The creation of spacious, dry and mulched rest areas within slaughterhouses.

The succession of slaughter operations offers a multitude of possibilities for direct contact (turning of the leather, etc.) and indirect contact (*via* equipment, men, etc.) between muscle masses and sources of contamination. Slaughter hygiene, therefore, consists of minimizing this contamination as much as possible. This is why, today, the skinning hygiene rests first on the cleaning and disinfecting practices of the materials, if possible between each carcass, and on the specialization of hands and tools (one hand was affected during the handling of the leather, the other during the handling of the carcass). Skinning in an upright position is desirable to minimize contamination of carcasses by soil flora in order to avoid cross-contamination by the leather of other carcasses. Pressure rinsing of the carcasses after evisceration makes it possible to eliminate the microorganisms which follow the flow, and to consequently allow for a decrease in the bacterial load of the carcass surface on certain precise sites. Lenahan et al. (2010) have shown that washing after

evisceration will reduce the microbial load on the surface of carcasses.

The harmonization of the cadence between the different phases of the slaughter line in order to avoid the overlapping of soiled and clean carcasses.

Lowering the carcass temperature as quickly as possible (refrigeration). However, care must be taken not to cause cryo-muscular shock (permanent contracture of myofibrils due to poor meat tenderness). This stage plays an important role in preserving the microbiological quality of fresh meats and determining their expiration dates.

Antimicrobial resistance of isolated bacteria

Widespread bacterial resistance is a serious risk to the whole population. This problem exists everywhere, especially in less developed countries due to self-medication (Rather et al., 2017). Food is the main agent which transfers resistant bacterium. This transfer can occur by saprophytic, commensal and pathogenic bacteria or by the presence of antibiotic residues (Founou et al., 2016). Twenty bacteria among those isolated during the assessment of the carcasses' microbial quality were used for the study of the antibiotic-resistance, including 10 strains of *E. Coli* and 10 *S. aureus* (Table 3). The study of susceptibility towards antibiotics was carried out by using the diffusion in a solid medium method. Mueller-Hinton was the medium used in accordance with the standards and the recommendations of the French Society of microbiology's antibiogram committee (SFM, 2018). To evaluate the antimicrobial resistance of isolated bacteria we have tested 9 antibiotics belonging to 9 families of the frequently used antibiotics in Morocco (amoxicillin, ciprofloxacin, gentamicin, erythromycin, benzylpenicillin, tetracycline, thiamphenicol, nitrofurantoin and the cotrimoxazoles) (El-Youbi et al., 2016). The results of antibiotic-resistance of bacteria isolated from Beni-Guil carcasses are presented in Table 3.

According to Table 3, the majority of the tested *E. coli* strains is resistant to more than three antibiotics (multi-drug resistant). The ten *E. coli* strains tested have shown a resistance of 50% to tetracycline and nitrofurantoin, while 100 % are resistant to ciprofloxacin and thiamphenicol. However, 100% of tested *E. coli* strains are sensitive to amoxicillin and 60% to gentamicin. As shown in table 3, *E. coli* strains have shown high resistance to the tested antibiotics. The highest resistances recorded were to ciprofloxacin (100%), thiamphenicol (100%), and nitrofurantoin (50%).



Table-3. Antibiotic-resistance of *Escherichia coli* and *Staphylococcus aureus* strains isolated from Beni-Guil's carcasses in eastern Morocco

Antibiotic	<i>E. coli</i> (%)			Criteria for <i>E. coli</i> (mm)		<i>S. aureus</i> (%)			Criteria for <i>S. aureus</i> (mm)	
	R	I	S	S	R	R	I	S	S	R
Amoxicillin	0	0	100	≥19	<19	NT	NT	NT	-	-
Ciprofloxacin	100	0	0	≥26	<24	100	0	0	≥21	<21
Gentamycin	0	40	60	≥17	<14	70	0	30	≥18	<18
Erythromycin	NT	NT	NT	-	-	0	0	100	≥21	<18
Benzylicillin	NT	NT	NT	-	-	80	0	20	≥26	<26
Tetracycline	50	0	50	≥18	<15	50	25	25	≥22	<19
Thiamphenicol	100	0	0	≥17	<17	85.71	0	14.28	≥18	<18
Nitrofurantoin	50	25	25	≥11	<11	100	0	0	≥13	<13
Cotrimoxazole	0	0	100	≥14	<11	0	0	100	≥17	<14

S: Higher critical diameter; R: Lower critical diameter; R: Resistant; I: Intermediate; S: Sensitive; NT: Not tested

Whereas, all tested strains of *E. coli* are sensitive to amoxicillin and cotrimoxazole. An intermediate prevalence of resistance to gentamicin is demonstrated (40%). The recorded resistance to ciprofloxacin (quinolone family) is due to the fact that it's currently the most important group of antibiotics. Their effectiveness is related to their low toxicity and especially to the absence of plasmidic resistance (Fofana, 2004). This high recorded resistance to this family of antibiotics should be considered as alarming since the quinolones are antibiotics of last resort against *Enterobacteria* which is multidrug-resistant. The evolutionary nature of resistance mechanisms, therefore, calls for caution. Additionally, the obtained results for resistance to nitrofurantoin, thiamphenicol and ciprofloxacin are higher than those reported by Ravaonindrina et al. (2015) in Madagascar. This difference can be attributed to the intensification level of livestock production, since in Madagascar, livestock intensification is undeveloped. A similar result was reported for the resistance to ciprofloxacin by Van et al. (2008) in Vietnam.

The multidrug resistance of *Staphylococcus aureus* isolated in our study concerns antibiotics commonly used in veterinary medicine, in particular, beta-lactam, cyclines, cotrimoxazole, aminosides, macrolides and furane which are also used in the treatment of different bacterial infections in humans. The obtained results show that 100% of *Staphylococcus aureus* strains are resistant to ciprofloxacin and nitrofurantoin. Thus, 85.71, 80, 70 and 50% are resistant to thiamphenicol, benzylicillin, gentamicin and tetracycline

respectively. However, 100% of *S. aureus* isolated are susceptible to erythromycin and cotrimoxazole. The highest resistance percentages were found for the following antibiotics: ciprofloxacin and nitrofurantoin (100%), thiamphenicol (85.71%) and tetracycline (50%). However, 100 % of tested strains are sensitive to erythromycin and cotrimoxazole. The reason behind this high resistance to quinolone and furan could be explained by the excessive use in Morocco of these two families of antibiotics for curative or preventive purposes on animals. The obtained results are higher than those reported by Mercier (2003) for *S. aureus* isolated from goat carcasses. Concerning erythromycin, our results are lower than those found by Tefera et al. (2019) for *S. aureus* isolated from sheep and goat carcasses, however, the recorded resistance to the phenicol family is higher than that reported by Tefera et al. (2019). Except for the macrolide family (Erythromycin), Fthenakis (1998) had reported similar microbial resistance to beta-lactam (74%), aminosides (70%), and cyclin (44%). Similar resistance was recorded against cotrimoxazole in comparison with the study of Hanson et al. (2011) on bacteria isolated from retail meat in Iowa.

The high rate of recorded antibiotic resistance based on this study, is mainly due to self-medication, as well as the use of extended-spectrum antibiotics, which cause the development of direct or indirect antibiotic resistance. These differences can be mainly explained by the negligence of the good use of practices and the intensification of animal husbandry. However,



bacteria in the natural environment can harbor resistance genes derived from the use of these drugs in humans and animals. In addition, this finding concerning the resistance to thiamphenicol (phenicol family) should be taken with consideration, since the latter is considered as a molecule that can cause fatal bone and irreversible medullar aplasia (Hammett-Stabler and Johns, 1998).

The spread of multi-drug resistance (MDR) among pathogens is a global health risk because of its public health and economic incidence. The development of drug resistance among foodborne pathogens is mostly due to the abusive and anarchic use of antibiotics indicating a lack of adherence to treatment guidelines, overdosage, use of extended-spectrum antibiotics and the use of preventive agents as feed additives. Cross-resistance has a chromosomal origin and it only concerns antibiotics that have the same mechanism of action, therefore having a common site of action. Thus, resistance to an antibiotic may be accompanied by resistance to other antibiotics of the same family. The tested bacterial strains have shown a resistance to multiple antibiotics, It's a case of MDR. The latter is defined as a single isolate resistant to three or more unique antimicrobial classes. A similar result was reported by several internationally prominent authors (Van et al., 2008; Hanson et al., 2011; El Allaoui et al., 2013; Ravaonindrina et al., 2015). Based on these results and the comparisons, it is quite evident that there is an increase of antibiotic resistance in the world, which represents a serious threat to humanity. This study could be a scientific basis upon which manuals for monitoring and controlling hygiene in Moroccan slaughterhouses can be established. Thus, the implementations of drug use guidelines for breeders to rationalize their usage, avoid self-medication, and alternate antibiotics to reduce the development of bacterial resistance. So it would, therefore, be useful to deepen this study by following the evolution of carcass surface contamination during the animals' preparation for slaughter and investigating the origin of this contamination by resistant bacteria in order to determine whether said contamination is endogenous or exogenous.

Conclusion

The objectives of this study were the assessment of the bacteriological quality and to study the antibiotic resistance of bacteria isolated from Beni-Guil PGI lamb carcasses. From the health perspective, all the

carcasses studied have a satisfactory quality (absence of pathogenic bacteria). Regarding the study of antimicrobial resistance, most of the tested strains were found to be resistant to antimicrobial agents, and they fall mostly into the critically important category, with a large percentage of multi-drug resistance isolates, which raises great concern for the health of consumers.

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