

Full Length Research Paper

Screening of antibiotics residues in beef consumed in Ouagadougou, Burkina Faso

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The anarchic use of antibiotics for therapeutic purposes or as a growth factor in Burkina Faso's breeding is the origin of their residues present in consumed meat. These antibiotics residues have health, technological and microbiological consequences. The objective of this first study is to highlight the antibiotic residues in meat consumed in Ouagadougou, Burkina Faso. A survey was carried out to make an inventory of antibiotics used for animal treatment intended for production and the consequences of such use. Thus, a total of 100 samples of bovine kidney were deduced from the refrigerating slaughter house of Ouagadougou aseptically to test the presence of drug residues with the Premi[®]Test kit and the microbiological method with *Geobacillus stearothermophilus* var. calidolactis ATCC 10149. According to this analysis, 31% of the kidney samples contained aminoglycosides, quinolones, macrolides and beta-lactam, sulfonamides and/or tetracyclines causing the zones of inhibition 3 to 15 mm. This reflects the anarchic use of antibiotics in Burkina Faso cattle breeding. Measures must be taken to ensure consumer safety and reduce the impact of these antimicrobials on selection of resistant pathogenic bacteria strains.

Key words: Consumed meat, antibiotics residues, Burkina Faso.

INTRODUCTION

Foodborne diseases caused by microbial agents, biotoxins and chemical pollutants constitute a serious public health problem (FAO/WHO, 2004). Among the chemical pollutants found in food, antibiotics residues occupy a prominent place (votimir et al., 2011). Serious foodborne infections of epidemiological proportions have been reported globally in the past decades, showing their importance both for

public health and social plan. Worldwide, consumers are increasingly concerned about these epidemics such as bovine spongiform encephalopathy, avian influenza etc. (Lantier et al., 2004). The anarchic (lawless) use of antibiotics for therapeutic purposes or as a growth promoter in lives in livestock is causing serious problems associated with the presence of these residues in food

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animals such as milk, meat, eggs and fish (Cately et al., 2003; Ben-Madhi and Ouslimani, 2009).

Antibiotic residues in animal source foods are responsible for the modification of the intestinal flora (Abidi, 2004; Chestnut and Stevens, 2005), allergies by beta-lactams (Fabre and Joye, 2000a; Fabre et al., 2000b) and antibiotic resistance (Gysi, 2006). These residues constitute an important source of potential toxicological risk to the consumer (Kabir et al., 2004; Persoons, 2011). Besides these health risks, the antibiotic residues in milk and meat are responsible for the inhibition of fermentation processes (Heeschen and Blüthgen, 1990). Indeed, fermentative bacteria are often inhibited by low dose of antibiotics which raises manufacturing accidents (absence of fermentation) (Oliveira et al., 2006). These accidents affect industries and manufacturing units of dairy and meat processing, causing huge economic losses (Brouillet, 2002; Oliveira et al., 2006). In most of West Africa countries and Burkina Faso in particular, misuse of antibiotics by farmers have been reported (Biagui, 2002). Burkina Faso has an important bovine livestock in perpetual growth, 8,233,845 heads of bovine in 2009 (MRA, 2009).

Antibiotics and other veterinary drugs are given anarchically to animals for therapeutic, prophylactic purposes, and as growth promoters. This misuse has led to a proliferation of illicit drugs and self-medication among farmers (MRA, 2009). Faced with this misuse of veterinary drugs and the lack of information about antibiotics in meat residues in Burkina Faso, this work was aimed at detecting the antibiotic residues in the meat consumed in Ouagadougou, Burkina Faso.

MATERIALS AND METHODS

Investigation of the use of veterinary drugs

In order to make an inventory on the use of antibiotics, investigation and census were carried out from May to July, 2012 at the "Direction Générale des Services Vétérinaires (DGSV)" of Burkina Faso, veterinary pharmacies and clinics and nearby breeders. Specific questions were asked about the recording mechanisms of veterinary drugs, the place of antibiotics in veterinary drugs registered, the place of tetracycline in antibiotics, antibiotics largely sold. In breeding, tetracycline usage is important about the breeders (MRA, 2011). There was also a point about the practice of self-medication by breeders, knowledge of the timeout and respect of that period.

Sampling

This study focused on oxen kidneys slaughtered in the refrigerating slaughter house in Ouagadougou, Burkina Faso from March to July 2012. Kidneys are routinely used for detection of antibiotic residues (tetracycline/oxytetracycline, enrofloxacin, ciprofloxacin) in beef as they allow an overall assessment of the presence of antimicrobial residues in all carcass (Cooper et al., 1998; Myllyniemi et al., 2000; Cantwell and O'keeffe, 2006). A total of 100 samples were collected immediately after slaughter, the sampling was done with a frequency of 7 kidneys week. The oxen's carcasses' were destined

to human consumption. An amount of 230 to 380 g of kidney were removed, collected in sterile bags and placed in an isothermal box at 4°C before being transported to the laboratory for freezing at -20°C and analysis.

Screening of antibiotic residues

The frozen samples were thawed immediately and the liquid therein was recovered for analysis. Two successive tests were conducted to check the effect of lysozyme on the detection of antibiotic residues. The exudation (liquid) from the kidney contains active lysozyme capable to inhibit the growth of microorganism (Pikkemaat, 2009; Merten, 2010). The first test was performed with the active lysozyme and the second with the inactive lysozyme. A volume of 1 ml of liquid from the kidney was heated at 75°C for 15 min to inactivate the lysozyme (Nouws, 2000). The samples underwent firstly an initial screening with the Premi® Test Kit (DSM Premi® Test, NETHERLANDS). Premi® Test is qualitative and can detect the family of beta-lactam antibiotics, sulfonamides, aminoglycosides, quinolones, macrolides and tetracyclines. Samples found positive in comparison to positive control using the test kit were subjected to the confirmation by the microbiological method (AFSSA, 2006) with *Geobacillus stearothermophilus* ATCC 10149. In fact Mueller Hinton Agar (Liofilchem Italy) was first sowed by *G. stearothermophilus* to the order of 10^5 spores/ml. This suspension was prepared by pure colonies emulsion in physiological saline (NaCl: 9 g/L of water) and adjusting the absorbance in a spectrophotometer at 625 nm to a value of between 0.08 and 0.1 (equivalent to 0.5 McFarland standard). Sterile Wattman paper disks impregnated with the liquid kidney to undergo a capillary test were deposited on the surface of the inoculated agar and incubated at 55°C. After 24 h of incubation, the diameters of the zone of inhibition were measured. While diameters of the zone of inhibition was greater than 3 mm, the samples were considered positive and the diameters were low at 1 mm; as they were negative about positive and negative control. Targeted antibiotics are originated from the family penicillin and tetracycline.

DATA ANALYSIS

Data were entered and analyzed with EXCEL 2003® and WIN EPISCOPE® 2.0. Software. The frequencies were calculated with a confidence interval (CI) of 95% and a margin of error of 5% (0.05).

RESULTS

Survey

Veterinary drugs' Market authorization (MA) in Burkina Faso

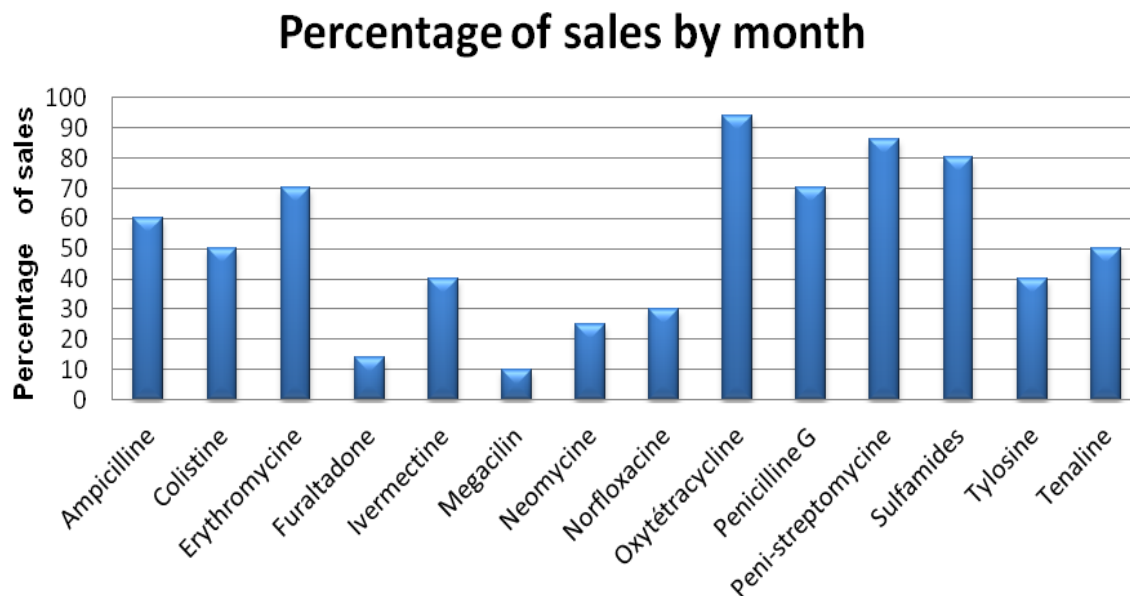
It is clear from this survey that the veterinary drugs are subject to the Market authorization (MA) in Burkina Faso. The registration process takes into account aspects such as speciality of pharmaceutical form, dosage and presentation.

Veterinary drugs sold in Burkina Faso

Among the veterinary drugs sold in Burkina Faso, antibiotics occupy an important place. Table 1 show that

Table 1. Distribution of veterinary drugs in Burkina Faso in 2009 (MRA, 2009)

Veterinary drugs	Quantity in ton and percentage
Antibiotics	2686, 95 (35%)
Antiparasitic	2456, 64 (32%)
Anti-inflammatory	383, 85 (5%)
Vitamins and others	2149, 56 (28%)
Total	7677 (100%)

**Figure 1.** Main antibiotics sold in pharmacies and veterinary clinics and their monthly sales proportion in Ouagadougou.

antibiotics account for 35% of the total quantity of veterinary drugs in Burkina Faso in 2009.

Antibiotics and sales proportions

Figure 1 shows the different antibiotics, proportions of sales and that commercialized. Oxytetracycline were most 94% sold followed by penicillin-streptomycin and the less (10%) used is megacilin.

Timeout's knowledge of breeders

No information and recommendation regarding the timeout are given to breeders who go to veterinary pharmacies to buy antibiotics. Regarding the good farming practices, among the 50 farmers who were interviewed 70% (35/50) said that they do not refer to a vet before treating their animals. 31% (11/50) of breeders admit to inject their animals.

Table 2. Prevalence of antibiotics residues without lysozyme inactivation.

Methods	Premi® test	Microbiological test
Positive	56 (56%)	59 (59%) [3 to 16mm]
Negative	44 (44%)	41(41%) [< to 3mm]

n= Number of samples; () = Percentage; [] = Minimum and maximum diameters of inhibitions zones measured.

Antibiotics residues

Microbiological and Premi® tests results without lysozyme inactivation shown respectively that 59 and 56% of samples were positive (samples that have caused zone of inhibition been greater than or equal to 3 mm for beta-lactam residues and/or sulfonamides, aminoglycosides, quinolones, macrolide and/or tetracyclines) (Table 2). Also, after lysozyme inactivation, microbiological and Premi® tests shown respectively that 31 and 32% of samples was positive (Table 3).

Table 3. Prevalence of antibiotics residues with lysozyme inactivation.

Methods	Premi® test	Microbiological test
Positive	32 (32%)	31 (31%) [3 to 9mm]
Negative	68 (68%)	69 (69%) [< to 3mm]

n= Number of samples; () = Percentage; [] = Minimum and maximum diameters of inhibitions zones measured.

DISCUSSION

Our results show that lysozyme, present in the sample can exert its antibacterial properties and cause false positives. So in reality, the prevalence of antibiotic residues in the meat consumed in Ouagadougou is 32%. In the case of the microbiological test the same table shows that 31% of the samples were positive. This confirms that the prevalence of antibiotic residues in meat consumed in Ouagadougou is actually 31%. When lysozyme is active we then notice a high percentage of positive whatever the used method. These results confirm the inhibitory action of lysozyme on the growth of *Geobacillus* in these samples where the meat juices did not undergo a step of lysozyme inactivation. In the case lysozyme is inactive the number of positive is relatively low regardless of the used method. So inactivation of lysozyme allows us to put aside false positives and reduce the severity of judgment that could be said about the prevalence of antibiotic residues in beef consumed in Ouagadougou.

In Burkina Faso there is a veterinary medicines registration and sale mechanism, but we observe a strong presence of street drugs in urban centers and especially in the villages. This makes the control system to be ineffective and exposes people to potential risks. Antibiotics accounted for 35% of the total volume of veterinary drugs in Burkina Faso and among these antibiotics tetracycline (oxytetracyclines) is the most used. These results corroborate with those found by Merten, (2010) where oxytetracyclines are the most used group of antibiotics in Nouakchott, Mauritania. In livestock markets, breeders ignore the concept of timeout. This is not surprising because in Burkina Faso most farmers are illiterate and 31% of those breeders practice self-medication (MRA, 2011). The works of Mitema et al. (2001) and Leopold et al. (2009) show that respectively in Kenya and Cameroon, self-medication is a common practice among breeders. Self-medication has become a habit for most Africans breeders. This practice nowadays prohibited in developed countries, could be at the origin of the selection of multidrug-resistant pathogens (Catory et al., 2003). These various results obtained from veterinary drugstores and the ones obtained from breeders attest the importance of the risk of finding antibiotic residues in beef, especially those from animals treated with antibiotics before slaughtering.

In Ouagadougou, 31% of consumed meat contains antibiotic residues. This high rate could be due to the lack of information and the practice of self-medication and the non-respect of timeout. This is worrying and must appeal the different actors of beef specialist about the poor quality of the meat in terms of chemical hazards. Comparable results were obtained in Mauritania by Merten's works in 2010. These works revealed, in fact that 58% of samples were suspects. Châtaigner and Stevens, (2005) reported that bovine meat, all races and all types of breeding combined, are heavily contaminated with antibiotic residues (about 42%). 31% positives found in Burkina Faso are below 54% positives found by Abiola et al. (2005) on antimicrobial residues in chicken liver and gizzard in the regions of "Dakar" and "Thies" (Senegal). This shows that Burkina Faso and all West African sub-region meat consumers are exposed to antimicrobial residues from animal source food. The identification of these residues reflects a misuse of antibiotics, essential veterinary therapeutic substances in livestock. Also uncontrolled use of antibiotics in breeding leads to the selection of resistant organisms with multiple adverse effects such as the increase of infections, mortality and decreased productivity (Klotins, 2006; Leopold et al., 2009).

Apart from these health risks, the presence of veterinary drug residues in animal source food is an important economic barrier to the international trade with must respect the new sanitary and phytosanitary rules of World Trade Organization (WTO). Lysozymes contained in the meat juice act as inhibitors to bacterial growth and may lead to false positives if they are not inactivated. These results corroborate with those found by Nouws, (2000) which stipulate that lysozymes contain bactericidal substances that can strongly inhibit the activity of bacteria in the meat juices. In Burkina Faso, as in most subregion the leaders of public health have not yet taken sufficient measures to protect consumers against chemical hazards associated with the presence of veterinary drug residues in meat. It is therefore necessary to implement a residues monitoring program.

Conclusion

The prevalence of antibiotic residues found in meat consumed in Ouagadougou in our study assumes a misuse of antibiotics by breeders and poor breeding practices in Burkina Faso. This miss use may contribute to the selection of resistant pathogens that pose a danger to public health. It should increase the awareness of policy makers on the need for strict application of antibiotic therapy in breeding. Regarding the risks associated with antibiotic residues, it is necessary to develop quality assurance programs for antibiotic residues in animal source foodstuffs. Using a chemical method such as HPLC may allow us to quantify and identify antibiotics in meat consumption in Ouagadougou.

Conflict of interests

The author(s) did not declare any conflict of interest.

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