Efficacy of different brands of penicillin-streptomycin against *Staphylococcus aureus*: the clinicians' myths and the realities

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Abstract

Background

Antibiotics must be safe, effective and have acceptable quality to use in both human and veterinary medicine. A cross-sectional study was conducted with the aim of evaluating *in vitro* efficacy of three brands of penicillin-streptomycin (penstrep) against *Staphylococcus aureus* isolated from dairy cow milk in Sebeta and Bishoftu, Ethiopia. A standardized questionnaire was also used to assess the knowledge, attitudes and practices (KAP) of veterinarians about brand antibiotics prescribing in dairy farms in the study area.

Results

A total of 43 *S. aureus* were isolated from 23 dairy farms. Thirty-three (81.4%) and 10 (23.3%) isolates were susceptible to brand A and intermediate susceptible to brand B, respectively, whereas only one isolate (2.3%) was intermediate susceptible to brand C. The isolates were also tested against the standard discs of penicillin and streptomycin, and the results showed that 42 (97.7%) and 27 (62.8%) of *S. aureus* were resistant, respectively. The KAP assessment showed penstrep was the most prescribed antibiotic in the dairy farms in the study area, followed by oxytetracycline and sulfa drugs. All veterinarians (100%, 30) agreed that antibiotics imported from Western countries perceived as having higher efficacy than those imported from eastern countries. Similarly, they preferred to use brand A, claiming better clinical improvements compared to the other two brands. The majority (86.7%) and more than half (53.3%) of the respondents agreed on the perception of overuse of antibiotics in veterinary clinics and dairy farms, respectively.

Conclusions

Penstrep Brand A has had better *in vitro* efficacy, while brands B and C have not been effective against *S. aureus* isolates. Moreover, this study revealed that a fixed combination dosage form of penicillin and streptomycin in brand A showed better efficacy towards *S. aureus* isolates compared to penicillin or streptomycin alone. The perception of the veterinarian about these brands agrees with the *in-vitro* antibacterial efficacy evaluation. The findings suggest that ineffective brands of penstrep are circulating in the local market, which constitute a potential danger to both human and animal health. Our findings warrant the need for stringent regulation and quality assessment criteria for imported veterinary antimicrobials.

Background

Antibiotics playing a significant role in reducing morbidity and mortality associated with common infectious diseases and; therefore, they have an important impact on both human health care and veterinary medicine (1). In food animals, antibiotics are used to control, prevent, and treat infection and enhance animal growth and feed conversion efficiency (2, 3). β-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, and sulfonamides are the most commonly used antimicrobials in food-producing animals (4). Penicillin, a β-lactam antibiotic and predominantly are active against Gram-positive bacteria (5), whereas streptomycin-sulfate, an aminoglycoside antibiotic are active against Gram-negative bacteria and Mycobacteria (6).

Penicillin-streptomycin (penstrep) is an antibacterial suspension comprised of fixed penicillin and streptomycin combinations. It is widely used in the control, prevention and treatment of infections in animals, especially in food-producing domestic animals because of their effective combined action against Gram-positive and Gram-negative bacteria (6).

Different antibiotics could potentially have variations in efficacy against the microorganisms they acted on. The differences in efficacy are not only between different groups of antibiotics but also within the same antibiotic with different brands or different producers; this could be due to the variation in the quality of active ingredients. However, one should be aware that low-quality medicines may not always be the result of problems at the manufacturing stage. This could be due to problems with packaging, transportation, storage conditions, and the distribution system (7, 8). Administering counterfeit drugs could result in therapeutic failure, toxicity, allergic reactions, drug resistance, prolonged illness, high cost of treatment and even mortality (8, 9).

The quality of drugs in less developed countries is questionable, although evidence is largely anecdotal. Studies published in *The Lancet* indicated that fake and poor-quality drugs are alarmingly common in some African and Southeast Asian nations (10). Recent studies have also reported that fake and low-quality medicines are prevalent in the developing world, where substandard and falsified medicines rise to almost 19% in African countries (11, 12). The World Health Organization (WHO) data have also suggested that around 10% of medications in poorer countries are fraudulent or substandard (13, 14). Reasons for poor quality include the widespread counterfeiting of medicines, decomposition of the active ingredient in drugs due to improper storage, and poor quality assurance during the manufacture of medicinal products (8, 15–17). Most developing and underdeveloped countries suffer from the direct and indirect effects of poor quality drugs at a high degree (16).

In Ethiopia, most livestock infections could be treated based on empiric therapy using the clinician's experience (18); however, resistance has been reported to the majority of essential antimicrobial agents currently approved for use in human and veterinary clinical medicine (19–24). Our field experience suggests that the prescription and utilization of antibiotics in both veterinary clinics and farms are often suboptimal in Ethiopia (18, 25, 26). This, combined with the variety of antimicrobial agents currently available, makes the selection of an appropriate agent a challenging task. This situation may force clinicians to depend more on data from *in vitro* antimicrobial susceptibility testing (AST). This highlights the significant role that diagnostic laboratories can play in providing information to improve veterinary services (27, 28).

Evaluation of some of the marketed products could give insight into the quality of products widely used in Ethiopia and could help to lay a foundation for future corrective measures. Studies evaluating the efficacy and quality of various brands of veterinary antibiotics in general and penstrep, in particular, are absent in Ethiopia.

Thus, this study was designed to evaluate the *in vitro* efficacy of different brands of penstrep against *Staphylococcus aureus* isolated from dairy cow milk and to assess the knowledge, attitudes and practices (KAP) of veterinarians regarding antibiotic quality and brand prescribing in dairy farms in Sebeta and Bishoftu, central Ethiopia.

Results

Bacterial isolation and antimicrobial susceptibility testing

Of 209 cow milk samples cultured, 43 (20.6%) *S.aureus* isolates were grown. A total of 43 *S. aureus* were tested for antibiotic susceptibility to three brands of penstrep relative to the standard discs prepared for penicillin (10µg) and streptomycin (10µg). As penstrep is a combination of two drugs, namely penicillin and streptomycin or dihydrostreptomycin. The measured value for the zone of inhibition of these three different brands was compared with the corresponding standards for penicillin and streptomycin (see additional file 1). The mean zone of inhibition recorded for all 43 *S. aureus* tested against the three brands was 16.86 mm (SD =3.16) , 9.92 mm (SD = 2.10) and 6.20 mm (SD = 2.24) for brand A, brand B, and brand C, respectively (Fig. 1). Extremely low mean of zone of inhibition was also recorded for standard discs of penicillin G (2.193, SD=2.21) compared with the streptomycin (10.84, SD = 3.56). A Kruskal-Wallis rank sum test showed that there was a statistically significant difference in zone of inhibition of *S. aureus* growth between different brands of penstrep and standard antibiotics (S and PEN) (Chi-squared = 158.0778, df = 4, p-value = 0.00).

Out of 43 *S.aureus* tested against three brands of penstrep, all of them (100%) were resistant to all brands as compared to the standard set for penicillin disc (10µg, Oxoid). However, (35/43) of the isolates were susceptible to brand A whereas 100% of them were resistant to both brand B and brand C compared to the standards set for streptomycin disc (10µg, Oxoid). The study result indicated that out of 43 *S. aureus* tested against the standard discs, 97.7% (n= 42) isolates were resistant to penicillin whereas 62.8% (n= 27) and 20.9% (n=9) isolates showed resistant and intermediate resistant to streptomycin, respectively (Table 1).

Table 1. Antibacterial activity of different brands of penstrep against S. aureus (n=43) isolated from dairy cow milk in Bishoftu and Sebeta

Farm antibiotic utilization practices

The assessment conducted on antibiotics commonly used in the dairy farms in the study area revealed that penstrep (100%, 23/23), oxytetracycline (78.3%, 18/23), and sulfa drugs (52.2%, 12/23) were the top three antibiotics mostly prescribed (see additional file 2). All dairy farms (100%, 23) used penstrep within the last 3 months period. Penstrep was the most commonly prescribed antibiotics to manage any septicemic conditions (100%), all suspected systemic bacterial infections (73.9%, 17/23), wounds (100%), mastitis cases that did not respond to intramammary antibiotic infusions (39.1%, 9/23), and as a prophylaxis during foot and mouth disease (FMD) and or lumpy skin disease (LSD) outbreaks (56.5%, 13/23), and as metaphylactic use in cases of FMD (39.1%, 9/23) and LSD (34.8%, 8/23) outbreaks. Oxytetracycline was the second most extensively prescribed antibiotics in the study dairy farms (87.0%, 20/23) to treat unknown diseases in non-pregnant and non-lactating cows. However, the long acting oxytetracycline formulation (20%) was the most commonly prescribed antibiotic to treat secondary bacterial complications in dairy animals infected with viral diseases, namely FMD and LSD; as prophylaxis use (78.3%, 18/23) and as metaphylaxis use (91.3%, 21/23). Sulfa drugs, the third most widely prescribed antibiotics in the dairy farms in the study area, were commonly prescribed to manage diarrhea in calves (52.2%, 12/23).

Knowledge, attitude, and practices (KAP) of the veterinarians about antibiotics use, quality and brand prescribing

Assessment of the attitudes of veterinarians about the antibiotics quality and brand prescriptions has revealed that all participants (100%, 30) agreed that antibiotics imported from western countries perceived as better quality than those imported from eastern countries . Similarly, all the participants perceived that some antibiotics of poor quality are available in the local market. However, although some veterinarians agreed that they prescribed antibiotics by international nonproprietary name (46.7%, 14/30) and by brand name (66.7%, 20/30), they also agreed that generic drugs perceived as equivalent to brand drugs (70%, 21/30) than generic antibiotics perceived as substandard drugs (30.0%, 9/30) (Table 2, see additional file 3).

Veterinarians' perception towards the country of antibiotics manufacturers revealed that all the participants agreed penstrep produced in the UK has better quality than those produced in China. Similarly, all the participants perceived that there were differences in clinical improvements among commonly used three different brands of penstrep. Though brand A of penstrep is relatively more expensive, clinicians preferred to use brand A (30, 100%); they were claiming that brand A has better clinical effectiveness than either of the other two brands (Table 2).

Veterinarian's knowledge about antibiotics use indicated that all veterinarians have good knowledge about major clinical indications of antibiotics (Table 2). However, 86.7% (26/30) and 53.3% (16/30) of them perceived that antibiotics are overused in veterinary clinics and dairy farms, respectively.

Table 2. Veterinarians' perception, practices, and knowledge of antibiotic quality, use and brand prescribing at Bishoftu and Sebeta

Discussion

Uses of antibiotics in the dairy farms for disease prevention and control are a common practice, however, concerns about their efficacy vis-à-vis antibiotic resistance are rising recently (25). The differences in efficacy among different brands of antibiotics constitute a potential danger to public health, as administering low efficacious antibiotics could enhance the emergence of antibiotic resistance (29). There is, however, paucity of information on the practices regarding antibiotic use in the dairy farms and the veterinarian's perception on antibiotics quality and brand prescribing. To our best knowledge, the efficacy of different brands of antibiotics used in veterinary medicine have not been evaluated in Ethiopia. Thus, in this study, we assessed antibiotics commonly used in the dairy farms, KAP of veterinarians about antibiotic uses, quality and brand prescribing, and evaluated the *in vitro* antibacterial efficacy of antibiotics most commonly prescribed in dairy farms against *S. aureus* isolates. To test the efficacy of antibiotics, three brands of an injectable dosage form of penstrep widely used in Ethiopia were used. The susceptibility test measures the ability of different brands of antibiotic to inhibit bacterial growth. It involves the *in vitro* testing by using disk paper diffusion technique and measuring their zones of inhibition (30).

The result of the farm antibiotic utilization practices study shows that penstrep was the most widely prescribed antibiotics in the studied dairy farms (100%), followed by oxytetracycline (78.3%) and sulfa drugs

(52.2%). Penstrep was prescribed in the study farms to manage suspected systemic bacterial infections, wounds, mastitis cases, and as prophylaxis and metaphylaxis during FMD and LSD outbreaks. Similar findings were reported by a study conducted in Ethiopia (31).

The Food and Drug Administration (FDA) of the US firmly suggests that generic drug products must contain the identical amounts of the same active drug ingredient as the brand name product (32). The result of the KAP study on antibiotics quality and brand prescribing show that the majority of the veterinarians in the study areas perceived generic drugs as equivalent to brand drugs (70%). However, although the availability of some brand veterinary antibiotics in the government veterinary clinic is very limited, all veterinarians preferred to prescribe drugs by brand names (100%) due to perceiving availability of poor-quality generic products in the local market. Similarly, the current study showed veterinarians perceived that antibiotics produced in the western countries has better in quality than the eastern countries. This study is in line with a report from Ghana indicating poor performance of 'Indian-made' products, but no product quality problems for European manufactured medicines sampled (33).

The knowledge of clinicians about correct indications of major antibiotics is very crucial to improve animal husbandry practices as irrational prescription of antibiotics may perpetuate drug resistance (31, 34). The current study shows veterinarians have good knowledge about major clinical indications of antibiotics. This study is in line with the findings reported by a study conducted in India (35). In contrast, there are reports of poor knowledge of veterinarians about antibiotic use in Ethiopia. The veterinrians perceived antibiotics are overuse in veterinary clinics (86.7%) than in the dairy farms (53.3%). Our previous study conducted in Ethiopia showed very limited antibiotic classes are widely prescribed and overused in veterinary clinics (18, 26).

Penstrep is a combination of two drugs, namely penicillin and streptomycin or dihydrostreptomycin. It has no specific standard zone of inhibition stated by CLSI or other standards. Moreover, *in-vitro* efficacy evaluation for different brands of penstrep against *S. aureus* has not been conducted so far. Hence, the study result in this discussion could not be compared with previous study findings. But the specific zone of inhibition for the different brands of this drug could be compared with both the penicillin and streptomycin standards as both drugs were combined in equal concentration, but not for brand A.

From the comparative efficacy evaluation of three brands of penstrep against the test organism, we found a considerable difference between brands which could be because of the difference in the quality of active ingredients present in the formulation or logistic systems. We found brand A was the most efficacious of all brands tested against *S. aureus* as 81.4% of the isolates were susceptible compared to streptomycin standard, which was 16.3%. The higher concentration of streptomycin found in brand A (250 mg/ml) compared to other brands (200 mg/ml) could be a reason for being the most efficacious above all the tested brands. 23.3% of the test isolate showed intermediate susceptibility against brand B; this drug found better efficacy next to brand A while the brand C showed only 1% of the isolate was intermediate susceptible to and it is a brand we found to be the least efficacious compared to all tested brands in this study. The efficacy of different brands of penstrep tested in this study was directly related to the capacity of the drug (percent of inhibition to the test organism, *S. aureus*) i.e., the more the drug to inhibit the test bacteria, the higher the efficacy of the drug (36).

The drugs found in combination to form penstrep, i.e. penicillin and streptomycin, were found with less effect on the test organisms. For instance, penicillin showed 100% resistance and streptomycin showed 62.3%

resistance. But the combined formulation of these two drugs can result in a drug with better efficacy which was seen in a brand A. This is due to synergistic drug effect (nature) of penicillin and streptomycin (37).

In this study, we observed differences in efficacies of three brands of penstrep available on the Ethiopian market. This could be due to the difference in their manufacturing process and or amount of active ingredient of the drugs (8). The difference in the quality could create a significant difference on the price of drugs which mostly related with the efficacy and quality of the drugs i.e. drugs with lower price were reported to have lower efficacy and drugs with higher price found with better efficacy (38). This agrees with our current study. For instance, on this study, brand A, with a price of 120.00 ETB per a bottle (100 ml) was the first ranked efficacious drug (81.4% susceptibility of *S. aureus*) as compared to other brands tested. Brand B with a price of 80.00 ETB, was the second-ranked brand in efficacy (23.3% intermediate susceptibility of *S. aureus*) while the brand C, with a price of 78.00 ETB was found the least efficacious brand (1% intermediate susceptibility of *S. aureus*).

In the present study, prescriber's perception towards the country of antibiotics manufacturer show that all veterinarians perceive penstrep produced in the UK has better quality than those produced in China. Similarly, all clinicians experienced differences in clinical improvements of animals being treated with different brands of penstrep. Accordingly, clinicians preferred to use brand A (100%); they were claiming that brand A has better clinical effectiveness than either of the other two brands of penstrep. This study is in line with the observed differences in *in-vivo* efficacy study of the three brands on *S. aureus* isolated from dairy cow milk.

The differences in efficacies among different brands of some antibiotics constitute a potential danger to health. Administering counterfeit drugs have known to result in therapeutic failure, toxicity, allergic reactions due to their content, drug resistance, prolonged illness, inflated cost of treatment and even mortality which all can directly or indirectly influence the public health. Most developing and underdeveloped countries were reported suffering from the direct and indirect effect of inferior quality drugs at a high degree (10). This directly related to most society found in such country can afford (highly use) low-cost drugs. As observed from this study brands of drugs with low efficacy were imported or bought at a low price. Moreover, we observed that the livestock sector of the country imports drugs of least price based competitive bid system. Accordingly, brand(s) with the least price antibiotics are commonly available in the government veterinary clinics, but private clinicians prefer to use well-known brands. So, for the current study it is very straight forward to predict the effect of overuse of least price brand(s) of antibiotics. This can also be one of the reasons that alarmingly increasing of drug resistant strains in resource limited setting countries.

Conclusions

Penstrep was the most widely used antibiotic in the dairy cattle farms and widely available in different brands on the Ethiopian veterinary drug markets. Both questionnaire survey and *in vitro* antibacterial activity test results showed there were evident differences in efficacy among different brands of penstrep. Brand A was found relatively more effective against the test organism. A fixed combination dosage form of penicillin and streptomycin in brand A showed better efficacy towards *S. aureus* than the penicillin or the streptomycin alone. The observed differences in efficacy of the three brands could be believed to be originated from the manufacturing process and internal composition (active pharmaceutical ingredients) or logistic systems. This

constitute a potential danger to animal health and welfare. Veterinarians' perception towards prescribing brand antibiotics agrees with the *in vitro* antibacterial efficacy evaluation findings.

The current study suggest that ineffective brands of penstrep are circulating in the local market, that constitute a potential danger to both human and animal health and could result in therapeutic failure, AMR, prolonged illness, the high cost of treatment and even mortality. The current study was limited to questionnaire and *in vitro* antimicrobial activity assay against *S. aureus*. The ZI for brands of penstrep was also interpreted using penicillin and streptomycin, which requires establishing a new value for penstrep. Hence, large scale studies on other bacteria, comparative animal studies, and quality assay for active pharmaceutical ingredient (API) of different brands of penstrep should be undertaken.

Methods

Description of the study area

The study was conducted in two areas in central Oromia, namely Bishoftu and Sebeta towns. Bishoftu town is located in East Shewa zone of the Oromia regional state, and the area is located at a latitude and 40°E longitude of 8°45'N 38°59'E at an altitude of 1920 m above sea level in central high lands of Ethiopia (39). Sebeta (Oromo: *Sabbataa*) town is located in the Oromia special zone surrounding Finfinne (Addis Ababa) of the Oromia region, Ethiopia; approximately 25 km southwest of Addis Ababa with a latitude and longitude of 8°54'40"N 38°37'17"E and an altitude of 2,356 m above sea level (40). Farmers in the vicinity of Bishoftu and Sebeta practice a mixed crop and livestock farming system (41).

Study design and sampling strategy

A cross-sectional study was conducted from February to May 2015 to isolate *S. aureus* from milk collected from randomly selected lactating dairy cows found in smallholder dairy farms of Bishoftu and Sebeta towns. A convenience sampling strategy was used to select 23 smallholder dairy farms (14 from Bishoftu and 9 from Sebeta). A total of 209 randomly selected lactating dairy cows, 114 from Bishoftu and 95 from Sebeta, were used for sampling.

A pretested questionnaire (see additional file 4) (translated to local languages; Afaan Oromo and Amharic) was also used to assess the type of antibiotic commonly prescribed in the study dairy farms (n= 23) by interviewing veterinarians or farm animal health professionals (if getting contacted) or a farm manager or farm owner or farm supervisor, by auditing the medicines or empty bottles found in the farms, and by collecting data from the patient case book available in each of the selected dairy farms at the study area. We also assessed professional's perception about brands of antibiotics and their prescribing practices of penstrep, the antibiotics commonly used in the dairy farms and at veterinary clinics in the study areas. The veterinarian's perception of antibiotic quality and clinical efficacy in comparison with brand antibiotics, country of manufacturer, and knowledge of antibiotic and its use, were assessed. Both private and government employee clinicians were selected for the interview. A total of 30 volunteer veterinarians who are practicing drug prescribing in the government and or private veterinary clinics or rendering ambulatory private clinical services in the study area were included in the study.

Following the recommendations by Newton et al (2009) guidelines for field surveys of quality of medicines (42), three different brands of penstrep were collected from randomly selected veterinary pharmacy located in the Bishoftu town. All three brands (see Table 3) were legally registered and approved for use in veterinary medicine in Ethiopia. The brands were selected by surveying for their availability in the veterinary clinics and or pharmacies/drug stores in the study area, and by assessing the most widely prescribed brands by veterinary clinicians and the most commonly found in the smallholder dairy farms. All the selected products were evaluated and passed the recommended assessment for pharmaceutical product characteristics: drug name, company name, country of production, batch number, legal permission, preparation, color inspection, manufacturing and expiry dates, and availability of label inserts (43).

Table 3. Characteristics by strength, country of origin and average price of brands of penstrep used for efficacy evaluation against S. aureus.

Laboratory examinations

The isolation and identification of *S. aureus* from the dairy milk samples were done following the Standard Operating Procedures recommended for microbiological technique (44). Then, the three brands of penstrep, mentioned in table 1, were used for efficacy evaluation. Both the microbiological analysis of the sample and efficacy evaluation were performed at Addis Ababa University College of Veterinary Medicine and Agriculture (AAU-CVMA), Bishoftu.

Preparation and impregnations of the antibiotic disks

The standard commercial disk for both penicillin (10 units) and streptomycin (10µg) were obtained commercially (Oxoid, UK). Known volume of different brands of penstrep was diluted at the time of disc preparation using sterile distilled water to obtain the working solution equivalent to the concentrations of the commercial standard disks. Tubes filled with sterile distilled water were used to make dilution till the concentration of the antibiotic solutions reaching10µg (20 µl) (45).

The sterile disks were placed in petri dishes and a fixed volume of 20µl or 0.02ml solution of each prepared brands of penstrep was loaded on each disc one by one using a pipette.

The impregnated discs were arranged in separate plates and dried by placing them in an incubator at a temperature of 37°C for 2 hours (45).

In-vitro antimicrobial susceptibility testing

The efficacy of the prepared antibiotics was tested against the test organism using recommended test protocols for the Kirby-Bauer disc diffusion method. In this study, a day-old young subculture of the test organisms *S. aureus* (n = 43) isolated from cow milk were used. The antibiotic susceptibility patterns of the isolates to different brands of penstrep were evaluated using the agar-disk diffusion method on MHA (5,30,46).

The inoculums were prepared from the cultures and were matched for turbidity with 0.5 McFarland suspension, and spread onto an MHA plates. The prepared antibiotic discs were aseptically placed on the inoculated agar plate along with the commercially available discs for comparison of the efficacy of the prepared discs. The plates were then incubated at 37 °C overnight and then measured for a diameter of the zones of inhibition (5), which is related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone directly correlates inversely with the MIC of the test bacterium. However, this depends on the concentration of the antibiotic in the disk and its infusibility (47).

Interpretation of disc diffusion testing results was performed following the Clinical and Laboratory Standards Institute (CLSI). For the three brands of penstrep, streptomycin 10µg and penicillin-G 10-unit disks were used as a standard disc. Finally, the diameters of the zone of inhibition around the disks were measured to the nearest millimeter using a digital caliper, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of CLSI (2015) and Magiorakos et al (2012) (48,49).

Statistical analysis

Data were entered into a Microsoft Excel dataset created specifically for the study using Microsoft Office 365 and statistical analyses were conducted using RStudio software Version 3.4. Categorical variables were expressed by proportions and their significance was assessed, when appropriate, using Chi-square (χ^2) test or Fisher's exact test. This is used to test potential associations between categorical variables and *P*-value of ≤ 0.05 was considered as statistically significant.

Questions were used to assess veterinarians' knowledge about antibiotics and their use (n= 6) and perception about veterinary antibiotics quality and brand prescribing (n = 9) (The results of the antibiotic knowledge and perception about veterinary antibiotics quality and brand prescribing questions were expressed by the values of either 0 (not correct) or 1 (correct) or on a five-point Likert scale "Strongly agree/agree" or "Strongly disagree /disagree." The percentage of correct answers for each knowledge questions and the statements Strongly agreed/Agreed by the respondents were calculated.

Continuous variables were expressed by means and standard deviations (SD) and assessed for statistical significance using Kruskal-Wallis chi-square test. Descriptive statistics were computed to summarize a data set for efficacy evaluation of different brands of penstrep. The results of the zone of inhibition recorded by the tested antibiotics and interpreted as an intermediate resistance (IR) was expressed by the value of resistance (R).

Abbreviations

AMR, Antimicrobial resistance; Brand A, Pen&strep (Norbrook); Brand B, Penstrep (Chengdu Quiankun); Brand C, Pro&strep (Hebei Yuanzheng); CLSI, Clinical and Laboratory Standards Institute; ETB, Ethiopian birr; FMD, Foot and mouth disease; KAP, Knowledge, attitudes and practices; MIC, minimum inhibitory concentration; LSD, Lumpy skin disease; MHA, Mueller Hinton Agar; *S. aureus*, *Staphylococcus aureus*; UK, United Kingdom; ZI, zone of inhibition.

Declarations

Ethics approval and consent to participate

This research was approved by the College of Veterinary Medicine and Agriculture Institutional Research Ethics and Review Board Committee. The study purpose was explained to the farm owners and other participants, the consent form was read out to them, and those who agreed were involved in the study. Confidentiality of the participants was kept using unique codes.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

TBT involved in the design of the study, questionnaire data collection, antibiotic disc preparation, antimicrobial susceptibility testing, data organization and analysis, and manuscript drafting and revision; AG collected sample, conducted bacterial isolation, identification and antimicrobial susceptibility testing, and drafted the manuscript; AFB, Tafese BT, DN and FR involved in the design of the study and manuscript revision; and all authors read and approved the final manuscript.

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Tables

Table 1. Antibacterial activity of different brands of penstrep against S. aureus (n=43) isolated from dairy cow milk in Bishoftu and Sebeta

Drugs tested	Susceptibility of the isolates		
	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin (P)	43 (100.0)	0 (0.0)	0 (0.0)
Streptomycin (S)	27 (62.8)	9 (20.8)	7 (16.4)
Brand A compared to (P)*	43 (100)	0 (0.0)	0 (0.0)
Brand A compared to (S)#	4 (9.3)	4 (9.3)	35 (81.4)
Brand B compared to (P)*	43 (100)	0 (0.0)	0 (0.0)
Brand B compared to (S)#	33 (76.7)	10 (23.3)	0 (0.0)
Brand C compared to (P)*	43 (100)	0 (0.0)	0 (0.0)
Brand C compared to (S) #	42 (97.7)	1 (2.3)	0 (0.0)

Brand A, Pen&strep (Norbrook); Brand B, Penstrep (Chengdu Quiankun); Brand C, Pro&strep (Hebei Yuanzheng); n, number of isolates; %, percent; P, penicillin (10µg); S, streptomycin (10µg); * indicates the susceptibility test result for the three brands was compared to the standard set for penicillin; # indicates the susceptibility test result for the three brands was compared to the standard set for streptomycin. Intermediate resistance was considered as resistance.

Table 2. Veterinarians' perception, practices, and knowledge of antibiotic quality, use and brand prescribing at Bishoftu and Sebeta

Question	Participants agreed /total participants (%)
Perception of veterinary antibiotics quality and brand prescribing	
Agree that some antibiotics are of poor quality in the market	30/30 (100)
Antibiotics from western countries perceived as better quality than eastern countries	30/30 (100)
Penstrep imported from the western country (E.g., UK) perceived as better quality than the Eastern countries (E.g., China)	30/30 (100)
Generic antibiotics perceived as equivalent quality to branded antibiotics	21/30 (70.0)
Generic antibiotics perceived as substandard drugs	9/30 (30.0)
Prescribe antibiotics by international nonproprietary name	14/30 (80.0)
Prescribe antibiotics by brand name	20/30 (66.7)
Prescribe brands of penstrep	26/30 (86.7)
Variation in clinical improvements among brands of penstrep	30/30 (100)
Which brands of penstrep showed better clinical improvements?	
Pen & Strep (Norbrook)	30/30 (100)
Which brands of penstrep mostly prescribed?	
Pen & Strep (Norbrook)	30/30 (100)[a]
Penstrep (Chengdu Quiankun)	16/30 (53.3)[b]
Pro & Strep (Hebei Yuanzheng)	8/30 (26.7)[c]
Knowledge and perception of antibiotics and their use	
Penstrep indicated for both Gram-positive and Gram-negative bacterial infections	30/30 (100)
Oxytetracycline indicated for gastro-intestinal and respiratory bacterial infections	30/30 (100)
Sulfa drugs indicated for diarrheic cases	30/30 (100)
Antibiotics indicated for prophylactic use for severe viral cases	30/30 (100)
Antibiotic prescribing Practices	
Perception of antibiotic overuse in the dairy farms	16/30 (53.3)
Perception of antibiotic overuse in the veterinary clinics	26/30 (86.7)

[b,c] prescribed only when drug [a] was not available in the clinic.

Table 3. Characteristics by strength, country of origin and average price of brands of penstrep used for efficacy evaluation against S. aureus

Brand name	Concentration (mg/ml)	Country of origin	Average price per bottle of 100ml in ETB ($USD)
Brand A	S(250mg/ml) [a] P(200mg/ml) [b]	UK	120 (5.89)
Brand B	S(200mg/ml) [a] P(200mg/ml) [b]	China	80 (3.927)
Brand C	S(200mg/ml) [a] P(200mg/ml) [b]	China	78 (3.829)

ETB, Ethiopian birr; S, Streptomycin; P, Penicillin; $ the average price based on the exact date of sample collection ($1 USD = ETB 20.3706) in the April 2015.

Figures

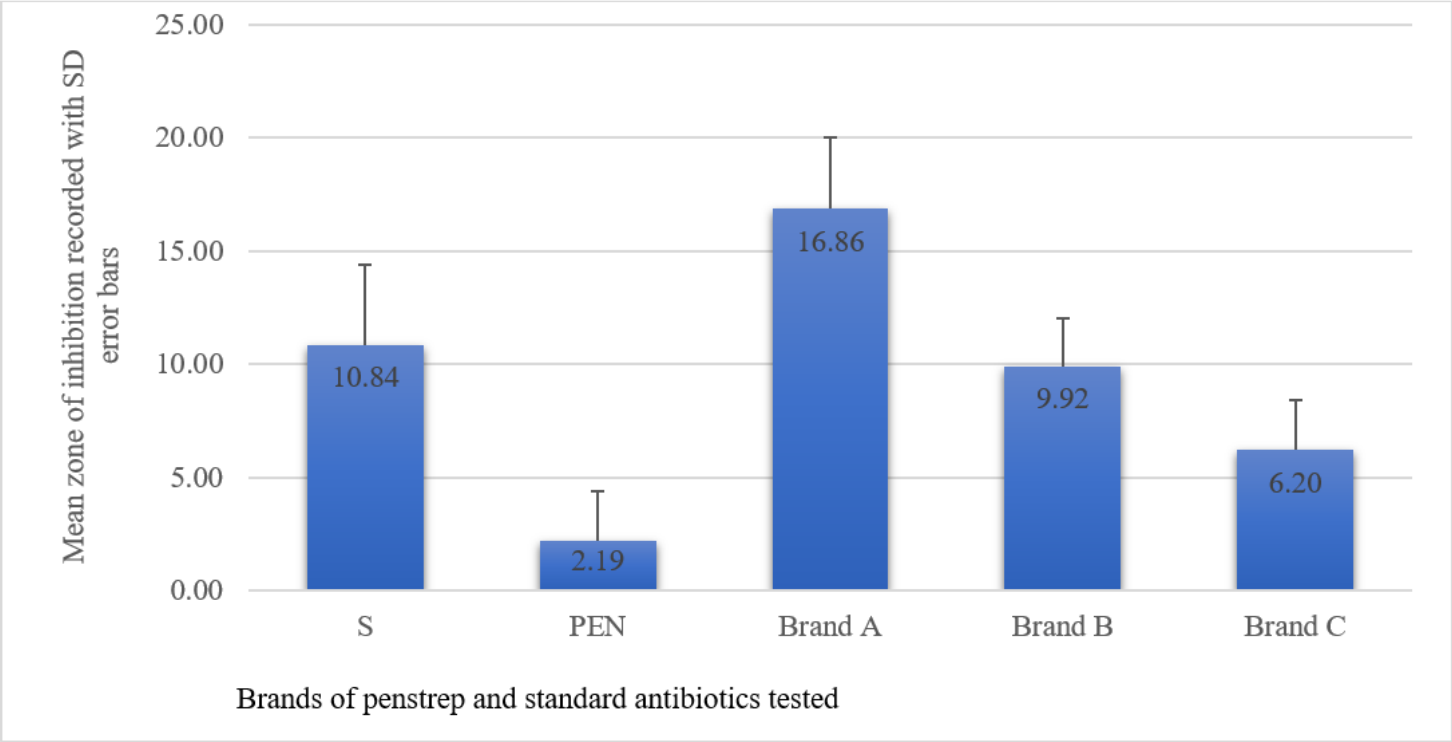

Figure 1

Zone of inhibition recorded by three brands of penstrep and standard discs of penicillin and streptomycin against S. aureus (n = 43) isolated from dairy cow milk in Bishoftu and Sebeta. S, streptomycin; PEN, penicillin G; and SD, standard deviation

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