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(RESEARCH ARTICLE)



Antibiotic susceptibility profile of bacteria isolated from packaged milk products sold in Sokoto, Nigeria: The resistant strains

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Abstract

Antibiotics are generally the drug of choice in the treatment of various infections both in humans and animals. But the overuse and misuse of antibiotics in animal production and human medicine have led to the development of vast amount of resistant organisms difficult to treat with antibiotics and thereby posing serious health complications. This study was conducted to examine the antibiotic profile of bacteria isolated from packaged milk products sold in Sokoto metropolis in order to determine possibility of resistant strains. Forty-six bacteria from packaged milk products were subjected to antibiotics susceptibility test using a single disc of 7 antibiotics by disc diffusion method following Clinical and Laboratory Standards Institute's guideline. Susceptibility result showed that high percentage of the bacteria were resistant to erythromycin (73.9%). However, very high percentages were susceptible to ciprofloxacin and tetracycline with 91.3% and 89.1% respectively. The finding of the study showed that packaged milk products sold in some areas in Sokoto metropolis contained resistant bacteria to commonly used antibiotics in the study area, therefore posing a serious health risk to the consumers. Good manufacturing practices need to be put in place to forestall any health problem as a result of consumption of contaminated packaged milk products.

Keywords: Antibiotic profile; Resistant strains; Milk products

1. Introduction

Antibiotic susceptibility is the growth-reactions of bacteria to antibiotics. It is being carried out in the laboratories to determine the particular antibiotic that will successfully treat a bacterial infection. Antibiotics are essential therapeutic materials for the treatment of bacterial infections. The emergence of antibiotic resistance poses serious health, social and economic problems because infections caused by antibiotic-resistant bacteria often fail to respond to standard treatments, thereby reducing the possibilities of effective treatment and increasing the risk of morbidity and mortality in serious diseases [1].

Antibiotics are used in various ways in animal production. They are reported to have been used in treating and controlling bacterial infections as well as in growth promotion [2]. In treating bacterial infections, the prolonged use of antibiotics often time at low dosage promotes antibiotics resistance among bacteria in the gastrointestinal tract of animals we feed on. As is the case in the Fluoroquinolone-resistant *Campylobacter* that have emerged as a result of misuse of Fluoroquinolone in poultry [3]. It has been reported that when contaminated food is consumed, the resistance gene from commensal bacteria would be transferred to other bacteria which include foodborne pathogens in the intestinal tract of humans. Some of the studies conducted by the Centers for Disease Control and Prevention (CDC) revealed that increase in antibiotics resistance in *Salmonella* strains studied was possibly as a result of the antibiotic use in food animals. Furthermore, it was ascertained that most infections caused by resistant strains are acquired from

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the consumption of contaminated food such as milk and meat products [4]. This increase was due to sequential acquisition of plasmids and transposons coding for drug resistance to a wide range of antibiotics such as ampicillin, chloramphenicol, gentamicin, kanamycin, sulphonamides, tetracycline and trimethoprim [5]. There is a postulation that the higher the prevalence of bacterial resistance in animal production, the greater the extent of transfer of antibiotics resistance from animals to humans [6]. Antibiotics have been reported commonly used in human medicine to treat bacterial infections but they are not supposed to be used against viral infections such as common cold, sore throats, and flu [7, 8]. The overuse and underuse of antibiotics have been reported to be the main cause of resistance among bacteria in general [8, 9]. The spread of resistant bacteria from animals to humans has been cited as the main factor in the development of resistant strains among human bacteria organisms [10]. The improper use of antibiotics in the hospitals together with the close contact among sick patients encourages the spread of resistant bacteria strains [11].

Several environmental stresses adopted in food preservation have been cited as capable of causing increase in bacterial resistance to antibiotics. A study reported an increase in antibiotics resistance in foodborne pathogens which include *S. aureus*, *E. coli*, and *S. typhimurium*, under sub lethal low pH or high sodium chloride stress. In another study, it was shown that high osmolarity and starvation regulates the expression of bacterial lipocalin, a protein which helps in bacterial adaptation to environmental stress and responsible for the dissemination of antibiotic resistance genes. Environmental stress improves plasmid transfer and plasmid numbers and thus increases resistance [12].

Socio-economic factors have been reported as impetuses to bacterial resistance among human bacterial isolates in developed and developing countries [13]. Easy access to antibiotics in developing countries as led to their overuse [14, 15] accounting for resistance rates of 90% among human bacterial isolates to tetracycline in West Africa where misuse of this group of antibiotics has been on for many years [16]. Underuse of antibiotics which has been stated to be an important cause of development of resistance is also a common practice in developing countries [14]. This is so because, in poor countries, patients mostly are unable to afford full course of their medicines to be cured of their sickness, they often time result to either buying fake drugs that are cheaper or taking under dose of genuine drugs. Problem of antibiotic-resistance would likely persist in Africa in as much as the use of antibiotics is not regulated and antibiotics are of substandard quality [14, 15]. The use of substandard antibiotics has been reported to have led to production of resistant pathogens during treatment even with correct diagnosis [14, 16]. In developed countries, overuse of antibiotics has been identified as major factor in the development of bacterial resistance. This includes many ways like prescribing broad spectrum antibiotics when bacteriological evidence shows that a narrower spectrum drug is the right choice, and prescribing antibiotics due to patient mounting pressure to have antibiotics even when the infection is viral [17, 18].

Evaluation of the bacterial quality of packaged milk products become apparent due to the high risk associated with consuming substandard or unhygienic milk containing pathogenic organisms; health complications associated with consumption of inadequately pasteurized milk products include serious infections that are hard to treat with antibiotics. This becomes clinically significant if organisms isolated from an assessed sample is resistant to conventional antibiotics. Thus, it can confer antibiotic resistance to the infected host while calling for production of an alternative drug [19]. Early detection of food contaminants has contributed greatly to safety of foods and thus to an improvement of public health [20].

Milk contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistant determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food borne pathogens has increased in recent decades [21]. Furthermore, lack of proper control on the use of antibiotics in human health and animal production has led to increase in antibiotic resistant milk-borne pathogens. The fluid or semi-fluid nature of milk and its essential nutritional chemical composition has made it an ideal culture media for microbial growth [22]. And it is because of this reason, milk and milk products are more prone to the harboring and multiplication of microorganisms. As milk and milk products are essential foods to all nations, the products must therefore be of high hygienic quality.

Despite the implication of milk and milk products as one of the ways by which antibiotic resistant microbes are being transferred to humans, data of antibiotic resistant microbes from milk is still scanty. To be able to have a clear picture of resistant strains of milk products and thereafter devise means of combating the menace, there is need to undertake a study on antibiotics susceptibility of bacteria isolated from milk products.

2. Material and methods

Antibiotic susceptibility study was performed with a single disk of 7 antibiotics using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [23]. An overnight culture of each

isolate obtained from packaged milk products from Sokoto Metropolis was prepared on nutrient broth and incubated at 37 °C for 18 hours. Dry sterile plates of prepared Mueller Hinton agar were inoculated with the standardized inoculums of 18 hours' culture of test bacteria isolates. Gram negative bacterial isolates were standardized to 10⁵ CFU/ml while Gram positive bacteria isolates were standardized to 10⁶CFU/ml [24]. After inoculation, plates were allowed to dry in sterile incubator at 37 °C before placing the sensitivity discs of various antibiotics aseptically in triplicate. Antibiotics impregnated disks (Oxoid, Ltd., England) used include: Amoxicillin/clavulanic acid (30 µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg) and Sulphamethoxazole/trimotoprim (25 µg). Plates were allowed to stay for one hour before incubating at 37 °C for 18 hours. The zones of inhibition were measured to the nearest millimeter using a transparent ruler. CLSI guideline was used to interpret the results and classified the bacteria into sensitive, intermediate and resistant bacteria. The intermediate bacteria were regarded as resistant bacteria to be on the safe side.

3. Results

The susceptibility testing of the bacteria isolates (n = 46) showed that 91.3% of the examined bacteria isolates were sensitive to ciprofloxacin, 89.1% to tetracycline, 73.9% to gentamicin, 71.7% to cotrimoxazole, 67.4% to chloramphenicol, 52.2% to amoxylin/clavulanic acid and 26.1% to erythromycin in descending order (Table 1).

Table 2 shows that bacterial isolates from the milk sample were resistant to a wide range of antibiotics. *Enterobacter spp* isolated was very highly resistant (100%) to chloramphenicol, ciprofloxacin, erythromycin and gentamicin with zero resistance to amoxylin/clavulanic acid, cotrimoxazole and tetracycline. *Samonella spp* were 100% resistant to amoxicillin/clavulanic acid, erythromycin and gentamicin. *E. coli* had highest resistance (71.4%) to erythromycin and zero resistance to cotrimoxazole and tetracycline. *S. aureus* were highly resistant (60%) to amoxicillin/clavulanic acid and erythromycin with low resistance (10%) to ciprofloxacin, gentamicin and tetracycline respectively. All the isolates, however, were having varying sensitivity to tetracycline.

Table 1 Antibiotics susceptibility profile of bacteria isolates from milk samples

Antibiotics	Percentage Susceptibility (n=46)
Amoxicillin/clavulanic acid	52.2
Chloramphenicol	67.4
Ciprofloxacin	91.3
Cotrimoxazole	71.7
Erythromycin	26.1
Gentamycin	73.9
Tetracycline	89.1

Table 2 Distribution of bacterial isolate's resistance based on zone of inhibition produced by test antibiotics (%)

Antibiotic	SAL (n=2)	SIG (n=4)	ECL (n=7)	SER (n=1)	PRO (n=2)	PSE (n=5)	ENT (n=1)	STA (n=2)	BAC (n=2)
AMC	100	75	28.6	100	50	40	0.0	60	35.7
CLO	50	50	42.9	100	50	0.0	100	40	14.3
CIP	0.0	0.0	14.3	0.0	0.0	0.0	100	10	7.1
COT	0.0	25	0.0	0.0	50	20	0.0	40	42.9
ERY	100	100	71.4	100	100	80	100	60	64.3
GEN	100	25	28.6	0.0	50	40	100	10	14.3
TET	0.0	25	0.0	0.0	0.0	20	0.0	10	14.3

Key:

n = number of the isolates, AMC = Amoxicillin/clavulanic acid, CLO = Chloramphenicol, CIP = Ciprofloxacin, COT = Cotrimoxazole, ERY = Erythromycin, GEN = Gentamycin, TET = Tetracycline, SAL = Salmonella spp, SIG = Shigella spp, ECL = *E. coli*, SER = Serratia spp, PRO = Providencia spp, PSE = Pseudomonas spp, ENT = Enterobacter spp, STA = *Staphylococcus aureus*, BAC = Bacillus spp.

4. Discussion

There is need for periodic surveillance of laboratory activities in order to monitor antibiotic resistance and its spread. This will help in gathering information needed in making policies that matter on antimicrobial resistance [25]. The current study on antibiotics susceptibility of bacteria isolates from yoghurts sold in Sokoto metropolis showed varying degrees of bacterial resistance. The order of susceptibility of the studied antibiotics generally was ciprofloxacin (91.3%) > tetracycline (89.1%) > gentamicin (73.9%) > cotrimoxazole (71.7%) > chloramphenicol (67.4%) > amoxicillin/clavulanic acid (52.2%) > erythromycin (26.1%). Majority of the bacterial isolates were susceptible to ciprofloxacin (91.3%) and tetracycline (89.1%) as shown in Table 1. Leibowitz [26] had similar result where he claimed that ciprofloxacin (94.5%) and tobramycin (91.9%) were equally effective in eradicating or reducing bacterial pathogens. In another work, Leibowitz [27] recorded 91.9% success in the treatment of bacterial keratitis using ciprofloxacin. The effectiveness of ciprofloxacin, a second generation antibiotic, might be attributed to low usage of the antibiotic in the study area. Similar scenario was observed for tetracycline because it was also found to be effective.

Okonko *et al.* [28] reported high bacterial isolates' resistance to amoxicillin, tetracycline and cotrimoxazole (60 to 100%) which is in contrast to the findings of this study where cotrimoxazole and tetracycline were having low resistance of 28.3% and 10.9% respectively. Another contrasting result was obtained by Ayandiran *et al.* [29] where resistance pattern of all isolated bacteria from poultry farms in Ibadan revealed that 77% were resistant to tetracycline.

Table 2 shows a wide range of antibiotic resistance among the bacterial isolates (0 – 100%). In the current study, *Salmonella* spp recorded 100% resistance to gentamicin and amoxicillin/clavulanic acid while *Staphylococcus aureus* recorded 60% resistance to amoxicillin/clavulanic acid. Resistance to gentamicin and amoxicillin/clavulanic acid has great health implications as these are antibiotics of choice in the treatment of bacterial infections. The high usage of gentamicin and amoxicillin/clavulanic acid in the study area may be the cause for their resistance. Antibiotic resistance of isolated bacteria from milk products may also be a reflection of the harmful effects of self-medication. Many microorganisms have been reported to be persistent in the environment and have been isolated from ground water [30] which could probably be used at times in the preparation of milk products. This could enhance the emergence and spread of bacterial resistance among people who might consume these milk products.

5. Conclusion

Results from this study showed that majority of the bacterial isolates were susceptible to ciprofloxacin and tetracycline while sizeable proportions were resistant to gentamicin and amoxicillin/clavulanic acid. Resistance to gentamicin is of great health concern. The results therefore emphasize the importance of antimicrobial sensitivity tests in determining suitable antibiotic in the treatment of infection.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest is disclosed by either of the authors.

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