



Original Research Article

Probiotic yoghourts sold in Accra: Potential public health issues arising from microbiological quality and safety

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ABSTRACT

Keywords

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Resistance,
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Quality.

The current study evaluated the microbiological quality and safety of twenty (ten local and ten imported) probiotic yoghourt samples sold in Accra, Ghana. Results of counts (CFU/ml) showed that, (30%) each of imported and local samples were below the recommended standard of $\geq 10^6$ CFU/ml while all (100%) pH values of both imported and local samples were within the recommended standard of ≤ 4.5 . All identified genera have some history of use (safe contact) as probiotics even though some have a longer history of use than others. The most frequently isolated bacteria from local samples was *Lactobacillus brevis* (40%) and *Bacillus licheniformis* (40%) while in the case of imported samples *Streptococcus thermophilus* (37.5%) was isolated most frequently. Only *Bacillus licheniformis* showed haemolysis, in local samples (25%) and in imported samples (50%). In all, out of 36 isolates identified in both local and imported samples, 30.56% were sensitive to all 11 antibiotics tested while the remaining 69.44% isolates showed Multi Drug Resistance (MDR) to three or more antibiotics. *Bacillus licheniformis* from both local and imported samples exhibited resistance to the most 5 antibiotics. In general, MDR to three antibiotics dominated the resistance patterns: 25% (Ery/Chl/Tet) and 16.67% (Chl/Tet/Pen) followed by five antibiotics (Amp/Chl/Tet/Pen/Flx) 22.22% and four antibiotics (Amp/Chl/Tet/Pen) 5.56%. Irregularities in quality and safety found in this study suggest that all is not well in the probiotics industry and this calls for serious attention to avert possible public health issues associated with consumption of probiotics.

Introduction

Demand for value-added dairy products particularly, probiotic yoghourts (yoghourts with added live microbes) have increased considerably (FAO/WHO 2006). Recent estimates of the market for overall probiotic

products of \$15.9 billion in 2008 is expected to grow at a Compounded Annual Growth Rate (CAGR) of 11.7% from 2009 to 2014 (Markets and Markets, 2010). This success of probiotics has led to development and

marketing of a broad range of probiotic products without adequate measures to guarantee safety and efficacy. For example, low counts (Brink *et al.*, 2005; Hamilton-Miller *et al.*, 1998) and presence of virulence traits including antibiotic resistance (Ashraf and Shah 2011; Teuber *et al.*, 1999) and haemolysis (Kashid and Ghosh, 2010) have all been reported in probiotic bacteria. Consumption of probiotics has particularly been linked to increase mortality in patients with underlying disease (Besselink *et al.*, 2008). In terms of quality, variable pH (Dave, 1998), and antagonism between various groups of organisms (Varadaraj *et al.*, 1993; Morris, 1991) have been noted in probiotic yoghourts.

Probiotic yoghourts specifically play a role in stabilization of the intestinal microflora by competing against pathogens (Gibson *et al.*, 1997), reducing lactose intolerance (de Vrese *et al.*, 2001), prevention of antibiotic-induced diarrhea (Pochapin, 2000), prevention of colon cancer (Wollowski *et al.*, 2001) and stimulation of the immune system (Isolauri *et al.*, 2001).

However, for the health benefits associated with probiotics to be realized, they are required to contain pure live mono or mixed cultures of “health promoting” bacteria at cell counts above the therapeutic minimum (10^6) (FAO/WHO, 2001; Robinson, 1987). Even though a broad variety of microbes are used as probiotics, the most commonly used microorganisms in foods for human consumption are *Lactobacillus* and *Bifidobacterium* species because they are Generally Regarded as Safe (GRAS), they are acid and bile tolerant, and they have the ability to adhere to intestinal cells (Saarela *et al.*, 2000). Evidence (FAO/WHO, 2002) however suggests that probiotic effects are strain specific and resolution of the taxonomy of bacterial species therefore

remains a key point to be clarified, since it is well known that different species belonging to the same genus may have different beneficial properties. Additionally, appropriate pH of the product and storage temperature is necessary to guarantee the shelf life of the product.

Unfortunately, whereas probiotics are often regulated globally as dietary supplements rather than as pharmaceuticals or biological products (FAO/WHO, 2001), they are not regulated at all in Ghana. There is usually no requirement to demonstrate safety, purity, or potency before marketing probiotics in Ghana. This can lead to significant food safety implications such as the spread of antibiotic resistance genes and/or pathogenic bacteria as well as inconsistencies in quality. Relevant information regarding the probiotic market and literature on probiotic yoghourts is however limited in Ghana. The objective of the current study was to investigate the potency and safety of probiotic yoghourts sold in Accra.

Materials and Methods

Sampling

Twenty samples of probiotic yoghourts were procured from supermarkets randomly selected in Accra for this work. Samples were stored on ice, transported to the laboratory and immediately analyzed or stored at 4°C until they were analyzed.

Isolation of lactic acid bacteria

Ten grams of drinking yoghurt sample was aseptically weighted into 90 ml sterile 0.1% peptone water in a 100 ml conical flask to achieve a 1:10 dilution. Tenfold serial dilution of each sample was then subsequently prepared using 0.1% peptone water as diluent. One milliliter (1ml) volumes of each diluted sample was then

added in duplicate to sterile Petri dishes and molten de Man Rogosa Sharpe (MRS) agar (45°C) (Oxoid, Basingstoke, UK) poured into the dish and mixed thoroughly. Another layer of uninoculated MRS agar was poured over the surface of the first layer after it was set to produce a layer-plate as described by Oxoid manual, (2002). Plates were incubated packed in a gaspak system (BBL GasPak System) to provide an atmosphere of CO₂ (microaerophilic condition). Incubation was done at 37°C for 48hrs.

Enumeration of isolates

Visible colonies on the surface of MRS agar (Oxoid) plates were counted after 48hrs on a colony counter. Colony Forming Units per milliliter of sample (CFU/ml) was calculated as described by Downes and Ito (2001) and recorded.

Identification of isolates

Isolates were purified by sub culturing single colonies on fresh sterile media for identification. Gram staining was performed for differentiation of morphology and Gram reaction of the isolates. Identification was done using Analytical Profile Index (API 20E, bio Mérieux) according to the user's instructions manual. The presence of undesirable spoilage or pathogenic organisms was checked by the catalase test and gas formation as described by Cheesbrough (2006). Growth of lactic acid bacteria (LAB) isolates at different temperatures (15, 37 and 45°C) was examined in MRS broth by incubating for 48 hours under the same conditions described above and checking for turbidity.

Assay for Sensitivity to Antibiotics:

Pure cultures of identified isolates were screened for possible resistance against selected commercially prepared antibiotics

discs (Oxoid) including Erythromycin (E-), Tetracycline (Tet-), Chloramphenicol (CHL-) and Penicillin (PEN-), Ampicillin (AMP-), Gentamycin (GEN), Ciprofloxacin (CRX-), Floxacillin (FLX-), Cefuroxime (CTX-), Centriaxone (CTR-) and Cotrimoxazole (COT). At least one isolate per identified species recovered from a given product was included for antibiotic susceptibility testing. The disk diffusion method described by Kirby *et al.*, (1966) was adopted and modified for this work. Inoculum was prepared from purified isolates on MRS agar using sterile wire loop and inoculating 1% sterile peptone water in McCartney tubes. The turbidity was adjusted to 0.5 McFarland standards and applied onto freshly prepared MRS agar using a wire loop. A sterile swab was then used to spread the culture on the media. The inoculated plate was allowed to dry for some few minutes after which sensitivity disks were applied to it using a sterile forceps. Zones of inhibition around sensitivity disks were measured using calipers following 24-48hr of incubation at 37°C in a CO₂ atmosphere (BBL GasPak System). Results were interpreted according to the cut-off levels proposed by Charteris *et al.* (1998).

Assessment of virulence factors

Ability of isolates to haemolyse red blood cells was ascertained by plating isolates onto Blood Agar Base (Oxoid, UK) supplemented with 5–7% of sterile defibrinated sheep blood. The results were noted for complete haemolysis as β -haemolysis, partial haemolysis (green discoloration around the colonies) as α -haemolysis, and with no haemolysis as γ -haemolysis.

pH Determination

Sub-sample aliquot were homogenized and

equilibrated from samples into clean labeled beakers and the pH determined in triplicates using a calibrated digital electronic pH meter.

Data analysis

A Statistical Package for the Social Sciences (SPSS) was used to analyze data.

Results and Discussion

Generally, viable bacteria survivors were realized from all samples of both imported and locally produced probiotic yoghourts on MRS agar at 48 hrs of incubation. Counts were from 3.8×10^3 to 5.5×10^7 for local samples and from 6.5×10^3 to 6.3×10^6 for imported samples. A summary of the results are presented in Table 1.

Results of counts (CFU/ml) showed that, 3 out of 10 samples (30%) each of imported and local samples were below the recommended standard of $\geq 10^6$. All pH values (100%) of both imported and local samples were within the recommended standard of ≤ 4.5 (Table 1). In terms of pH and counts (CFU/ml), no difference was therefore observed between local and imported samples (Table 1).

Bacterial isolation from 10 local and 10 imported samples yielded a total of 7 bacteria species (3 from local samples and 4 from imported samples). This 7 bacterial species occurred 36 times in all the samples and all isolates were identified to the species level. The details are presented in Table 2.

Lactobacillus brevis (40%), *Bacillus licheniformis* (40%) and *Lactobacillus acidophilus* (20%) were isolated from local samples whereas imported samples yielded *Lactobacillus brevis* (18.75%), *Bacillus*

licheniformis (12.5%), *Lactobacillus acidophilus* (31.25%) and *Streptococcus thermophilus* (37.5%) (Table 2). The most frequently isolated bacteria from local samples was *Lactobacillus brevis* (40%) and *Bacillus licheniformis* (40%) while in the case of imported samples *Streptococcus thermophilus* (37.5%) was isolated most frequently (Table 2).

Only *Bacillus licheniformis* showed haemolysis and this was observed in isolates from both local samples (25%) and imported samples (50%) (Table 2).

Antibiotic resistance was observed amongst some identified isolates against some of the antibiotics tested. In all, out of the 36 isolates identified in both local and imported samples, 11 (30.56%) were sensitive to all 11 antibiotics tested while the remaining 25 (69.44%) isolates showed resistance to three or more antibiotics. The results are summarized in Table 3.

Bacillus licheniformis from both local and imported samples exhibited resistance to the most antibiotics: isolates from local samples showed resistances to ampicillin (50%), chloramphenicol (50%), tetracycline (62.5%), penicillin (62.5%) and floxacillin (37.5%). In the case of imported samples, 50% showed resistance against ampicillin, 50% against chloramphenicol, 50% to tetracycline and 100% against penicillin (Table 3). *Lactobacillus acidophilus* from local samples showed resistance to erythromycin (50%), tetracycline (50%) and chloramphenicol (75%) whereas the same bacteria from imported samples was resistant to erythromycin (40%), tetracycline (60%) and chloramphenicol (60%) (Table 3). *Streptococcus thermophilus* from imported samples exhibited resistance against chloramphenicol (50%), tetracycline (33.3%) and penicillin (33.3%). All (100%)

of *Lactobacillus brevis* isolates from imported and local samples were 100% sensitive to all eleven antibiotics tested and all other bacterial species identified exhibited multiple resistance to 3 or more antibiotics (Table 3). Five isolates (13.89%) showed resistance to ampicillin, 4 (11.11%) to erythromycin, 14 (38.89%) to chloramphenicol, 13 (36.11%) to tetracycline, 9 (25%) to penicillin, and 3 (8.33%) to floxacillin. All (100%) isolates were sensitive to gentamycin, ciprofloxacin, cefuroxime, centriaxone, cotrimoxazole (Table 3).

Multiple resistances (resistance to 3 or more antibiotics) were shown by 69.44% of all isolates in both imported and local samples. The results are summarized in Table 4.

The maximum MDR registered was resistance to five antibiotics (Amp/Chl/Tet/Pen/Flx) (Table 4). This was exhibited by 22.22% of the 36 isolates identified and found mainly in local samples. In general, MDR to three antibiotics dominated the resistance patterns: 25% (Ery/Chl/Tet) and 16.67% (Chl/Tet/Pen). The forma pattern was found in isolates from both local and imported samples whereas the later pattern was exhibited only by imported samples (Table 4). Multidrug resistance was also shown by 5.56% of isolates to four antibiotics (Amp/Chl/Tet/Pen) and this was found only in imported samples (Table 4)

The current study found irregularities in microbial counts, safety concerns (presence of haemolytic and antibiotic resistant bacteria) in some isolates from both local and imported probiotic yoghourts sold in Accra in contravention with the FAO guidelines ((FAO/WHO, 2001). The guidelines states that probiotic yoghourts should contain at the time of consumption

only safe strains of probiotics at viable cell concentration of $\geq 10^6$ CFU/ml and a pH of ≤ 4.5 . The study found lower counts than recommended by the standard in 30% each of imported and locally manufactured probiotics tested (Table 1). This is in agreement with Hamilton-Miller *et al.*, (1998) and Brink *et al.*, (2005) who came out with similar findings in probiotic yoghourts. This observation of low viable counts may have been present initially, suggesting inadequate quality control procedures, or may have resulted from bacterial death during the period of shelf-life. Time-temperature tolerance is known to affect frozen foods including yoghourts often exposed to a variable temperature environment, e.g. during distribution or due to freezing/defrosting cycle in retail or home freezers. For consumers to realize any health benefits from such probiotics with counts lower than the therapeutic level of $\geq 10^6$, they will have to consume more than the average pot (150ml) per day (Hamilton-Miller *et al.*, 1998) or consume the product at a daily dosage of 10^6 to 10^9 cells (Lee and Salminen, 1995).

According to Klaver *et al.* (1993), one of the most constraining drawbacks associated with the use of dietary cultures in fermented milk products is the lack of acid tolerance of some species and strains. The acidity of the probiotic yoghurt (pH) can therefore affect microbial counts depending on the species and strains used. Furthermore, pH is also said to affect the flavor and general quality of probiotic yoghurt (QC in yoghurt manufacture) fortunately, this study found 100% of both local and imported samples to conform to this pH requirement of ≤ 4.5 (Table 1).

Assessment of the safety of a probiotics includes verification of the strains of microbes used as starter culture because

probiotic effects are strain specific (FAO/WHO, 2002). Unfortunately, the guidelines (FAO/WHO, 2002) recommend the use of specific bacteria (*Lactobacillus* spp and *Bifidobacteria* spp) only to the genus level. Additionally, genotypic 16S ribosomal DNA–based identification of bacteria is recommended as a useful supplement to phenotypic characterization to result in species-level identification of strains (FAO/WHO, 2002). The large number of bacterial species under LAB and *Bifidobacteria* together with the high cost of genotyping makes the routine assessment of the safety of probiotics using strains verification an expensive venture (Hummel *et al.*, 2007) especially for resource-limited regulatory authorities in developing countries.

This study could not verify product quality and/or safety from claims on labels as most of the labels of products either only indicated that probiotic culture was used or disclosed only the bacteria genus used. Identification of 36 isolates using API revealed that the most frequently isolated bacteria from local samples was *Lactobacillus brevis* (40%) and *Bacillus licheniformis* (40%) while in the case of imported samples *Streptococcus thermophilus* (37.5%) was isolated most frequently (Table 2). *Bifidobacteria* was not isolated from any of the samples. The reason may have been that media used did not support the growth of *Bifidobacteria* in agreement with Temmerman *et al.* (2002). All isolated genera have some history of use (safe contact) as probiotics even though some have a longer history of use than others. For example, no *Bacillus* spp probiotic has been granted the status of GRAS (Generally Regarded As Safe) by the FDA of the USA yet (Sanders *et al.*, 2003). Non-haemolytic activity and antibiotic sensitivity are considered as a safety prerequisite for the selection of a probiotic

strain for probiotic yoghourts (FAO/WHO, 2001; EFSA, 2004). When probiotic strains enter the gut, they interact with the native microbiota and gene transfer can occur. Probiotics might contribute to the transfer of antibiotic resistance genes to other commensal bacteria or pathogens present in the GIT resulting in the occurrence of large numbers of transferable resistance genes within the intestines. The release to the environment in the faeces would then enable an accumulation of drug-resistance genes that can survive in the absence of a selective pressure. Most importantly, when pathogens become resistant to antimicrobial agents they can pose a greater human health risk as a result of potential treatment failure, loss of treatment options and increased likelihood and severity of disease (CAC/GL 77-2011).

Assessment of virulent factors of isolates as a safety indicator revealed that, only *Bacillus licheniformis* showed α haemolysis (Table 2) as well as exhibiting resistance to the most 5 antibiotics (Table 3) , suggesting it may not be safe to use this strain in the production of probiotic yoghourts. This finding is in agreement with Kashid and Ghosh (2010) with respect to haemolysis and with Sorokulova *et al.*, 2008 with regards to multidrug resistance. Evidence indicates that *Bacillus licheniformis* has been reported in cases of food-borne diarrhoeal illness, toxin production and infant mortality (Mikkola *et al.*, 2000). Therefore, any use of it as a probiotic must follow a complete evaluation of virulence factors (Huynh *et al.*, 2005). Antibiotic sensitivity test using the disc diffusion method also showed that in all, out of the 36 isolates identified in both local and imported samples, 30.56% were sensitive to all 11 antibiotics tested while the remaining 69.44% of isolates showed multiple resistance to 3 or more antibiotics (Table 3). Other resistances were, *Lactobacillus acidophilus* from local samples showed

resistance to erythromycin (50 %), tetracycline (50%) and chloramphenicol (75%) whereas the same bacteria from imported samples was resistant to erythromycin (40 %), tetracycline (60%) and chloramphenicol (60%) (Table 3). Temmerman *et al.* (2002) obtained 68.4% multiple antibiotics resistance of probiotic isolates using the disc diffusion method. Evidence from Ashraf and Shah (2011) indicates that LAB are intrinsically resistant to many antibiotics and this may have accounted for the high percentage of MDR obtained in this study. Even though intrinsic resistance is said to be non transferable, it is difficult to distinguish this from transferable resistance especially if the resistance gene is not located on a plasmid. Furthermore there are no approved standards for the phenotypic or genotypic evaluation of antibiotic resistances in food isolates (Kastner *et al.* 2006; Danielsen and Wind, 2003). Yet, the role of LAB as reservoir of antibiotic resistance determinants with transmission potential to pathogenic species is now increasingly acknowledged (Teuber *et al.*, 1999; Marshall *et al.*, 2009; van Reenen and Dicks, 2011). This study however found all (100%) of *Lactobacillus brevis* isolates from imported and local samples sensitive to all eleven antibiotics tested (Table 3). The results also revealed that the antibiotics with the highest resistance was chloramphenicol (38.89%) followed by tetracycline (36.11%). Remaining resistances were in the order of 25% to penicillin, 13.89% to ampicillin, 11.11% to erythromycin and 8.33% to floxacillin. Resistances in tetracycline (26%), penicillin G (23%), erythromycin (16%) and chloramphenicol (11%) were observed from European probiotic products (Temmerman *et al.*, 2002). Evidence of Roberts (2005), Thaker *et al.* (2010), Devirgiliis *et al.* (2011) indicates that, the most frequently described antibiotic

resistance in food borne LAB is to tetracyclines and erythromycin and this is as a result of their extensive use as growth promoters in the '60s and '70s, (Wegener, 2003). Resistance of LAB to chloramphenicol, aminoglycosides, betalactams, lincosamides, quinolones, rifamycins, sulfon-amides and fusidic acid have however been attributed to their use in studies aimed at selecting food borne antibiotic resistance strains of LAB. The corresponding antibiotic resistance most likely arose in environmental bacteria through selection due to improper use in human and veterinary medicine (D'Costa *et al.*, 2006). This study observed that all isolates (100%) were sensitive to gentamycin, ciprofloxacin, cefuroxime, centriaxone and cotrimoxazole (Table 3). These antimicrobial agents may not be frequently used in animals and humans.

In terms of resistance patterns, MDR to three antibiotics dominated the resistance patterns: 25% (Ery/Chl/Tet) found in isolates from both local and imported samples and 16.67% (Chl/Tet/Pen) exhibited only by imported samples (Table 4). Multidrug resistance was also shown by 5.56% of isolates to four antibiotics (Amp/Chl/Tet/Pen) those are found only in imported samples and to five antibiotics (Amp/Chl/Tet/Pen/Flx) by 22.22% of isolates from local samples (Table 4). Antimicrobial resistance patterns in specific geographical locations may aid clinicians in choosing the appropriate antimicrobial empirical treatment. Besides dedicated antibiotic resistance genes, MDR has been suggested as an intrinsic mechanism that can contribute to antibiotic resistance of bacteria (Price *et al.*, 2006; Putman *et al.*, 2001). Therefore, MDR found in this study including antibiotics commonly used in human medicine is worrisome.

Table.1 Summary of microbial counts and pH of probiotic yoghurts

Local samples			Imported samples		
Sample ID	pH Values of Samples	Count (CFU/ml)	Sample ID	pH Values of Samples	Count (CFU/ml)
L1	4.07±0.03	1.29×10 ⁶	M1	4.14±0.02	6.5×10 [*]
L2	4.08±0.01	4.2×10 ⁷	M2	4.08±0.02	4.8×10 ⁶
L3	4.34±0.02	4.1×10 ⁷	M3	4.11±0.02	5.0×10 ^{2*}
L4	4.06±0.03	3.8×10 ^{3*}	M4	4.23±0.02	3.36×10 ⁶
L5	4.19±0.04	4.0×10 ⁶	M5	4.07±0.03	5.1.0×10 ⁶
L6	4.21±0.03	2.7×10 ⁶	M6	4.10±0.02	1.18×10 ^{2*}
L7	4.07±0.03	3.6×10 ^{4*}	M7	4.29±0.04	3.7.0×10 ⁶
L8	4.22±0.02	3.8×10 ⁷	M8	4.06±0.02	6.3×10 ⁶
L9	4.19±0.03	6.3×10 ^{4*}	M9	4.07±0.02	3.12×10 ⁶
L10	4.14±0.40	5.5×10 ⁷	M10	4.15±0.03	4.8×10 ⁶
Standards	≤4.5				≥ 10 ⁶

N=3, pH values are means±standard deviation and counts are means. *Values are below recommended standard.

Table.2 Identified isolates and their potential virulence traits

Identified isolate	Occurrence	Percentage Haemolytic		
		α	β	γ
Local samples				
<i>Lactobacillus brevis</i>	8(40%)	0	0	8(100%)
<i>Bacillus licheniformis</i>	8(40%)	2(25%)	0	6(75%)
<i>Lactobacillus acidophilus</i>	4(20%)	0	0	4(100%)
Imported samples				
<i>Lactobacillus brevis</i>	3(18.75)	0	0	3(100%)
<i>Bacillus licheniformis</i>	2(12.5)	1(50%)	0	1(50%)
<i>Lactobacillus acidophilus</i>	5(31.25)	0	0	5(100%)
<i>Streptococcus thermophilus</i>	6(37.5)	0	0	6(100%)

Table.3 Antibiotic resistance of isolates against eleven tested antibiotics

Taxa (No of spp Identified)	Antibiotics										
	AMP	GEN	ERY	CHL	TET	CRX	PEN	FLX	CTX	CTR	COT
Local samples											
<i>Lactobacillus brevis</i> (8)	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillus licheniformis</i> (8)	4 (50%)	0	0	4 (50%)	5 (62.5%)	0	5 (62.5%)	3 (37.5%)	0	0	0
<i>Lactobacillus acidophilus</i> (4)	0	0	2(50%)	3(75%)	2(50%)	0	0	0	0	0	0
Imported samples											
<i>Lactobacillus brevis</i> (3)	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillus licheniformis</i> (2)	1(50%)	0	0	1(50%)	1(50%)	0	2(100%)	0	0	0	0
<i>Lactobacillus acidophilus</i> (5)	0	0	2 (40%)	3 (60%)	3 (60%)	0	0	0	0	0	0
<i>Streptococcus thermophilus</i> (6)	0	0	0	3(50%)	2(33.3%)	0	2(33.3%)	0	0	0	0

Table.4 Occurrence of Multiple Drug Resistance (MDR) in isolates and their profile

No. of antimicrobials Resistant to.	Antimicrobial resistance pattern	Occurrence (O) (% Occurrence= O/36x100)
Local samples		
3	Ery, Chl, Tet	4 (11.11)
5	Amp, Chl, Tet, Pen, Flx	8 (22.22)
Imported samples		
3	Ery, Chl, Tet	5 (13.89)
	Chl, Tet, Pen	6 (16.67)
4	Amp, Chl, Tet, Pen	2 (5.56)

In conclusion, irregularities in quality and safety found in this study suggest that all is not well in the probiotics industry. This calls for serious attention by all stake holders' particularly regulatory agencies. Instead of conferring health benefits,

probiotics may serve as a potential hazard of pathogenic food borne bacteria as well as antimicrobial resistant bacteria that may have public health implications.

This study recommends that the every starter culture should be tested for safety before being used for commercial production.

Conventional probiotic starter cultures should always be used.

There is the need to address the needs of different age groups and propose adapted probiotic formulations for them.

Investigate effect of probiotics on new health concerns.

For probiotic supplement users that are attempting a major "internal cleansing," on the advice of alternative medical sources, an incremental process is recommended where small occasional doses are tried at first.

Trying familiar strains of bacteria is recommended as a first stage.

A sudden large dose may cause digestive upset.

Using the more traditional advice from the European Food Safety Authority (EFSA) and Fergus Shanahan, the consumer should adopt the attitude of "let the buyer beware," and conduct significant research on the reliability and reputation of the company producing the probiotic, before purchasing or ingesting anything from that company.

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