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# Evaluation of bacterial contents, package labelling and antimicrobial activity of some commercial probiotic products available in local market

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**ABSTRACT**: The global use of probiotic products has been increasing steadily. These products are therapeutically intended for the prevention or treatment of various diseases. Commercial probiotic products are diverse, however no local or international regulations are applied to control the quality of these products. Many international studies have shown a scarcity of probiotic products that comply with the international guidelines. In Iraq, there are no previous studies that have looked at probiotic products from this scope. Therefore, the aim of this study was to assess whether the bacterial contents and package labels of some commercial probiotic products were correct. In addition, the study aimed to evaluate the *in vitro* antimicrobial activity of the isolated probiotics. Eighteen probiotic products were purchased from local community pharmacies within 7-month period. Bacterial contents were counted using culture and count method. Packages' labels were checked for contents and spelling accuracy. Antimicrobial activity was performed using conventional well-diffusion assay. Half of the eighteen products purchased from local pharmacies, did not fulfill the taxonomy and nomenclature of bacteria. 7 products (38.8%) demonstrated positive growth on culture media and none of them matched the labelled bacterial counts on their packages. Of these 7 products, it has been found that the 24 h-spent culture of product-1 was the only one that demonstrated the ability to inhibit the growth of *Staphylococcus aureus in vitro*. These findings necessitate the need for quality and efficacy control of these fairly expensive products. The effect of packaging and storage on the efficacy of these commercial products should also be taken into consideration.

**KEYWORDS**: Antimicrobial effect; bacterial count; label accuracy; probiotics; quality control; *Saccharomyces boulardii*.

## 1. INTRODUCTION

Probiotics are defined as organisms that live in co-operation with the host tissue and when administered alive in enough concentration, can beneficially influence the health of the host [1, 2]. The definition complies qualitative and quantitative requirements to achieve the potential health effect of probiotics. The field of probiotics is an attractive approach as preventive and/or therapeutic modality for many ailments in human and animals. This has largely been attributed to their advantages of being Generally Recognized as Safe (GRAS) and to minimize the spending of antimicrobial agents [3]. The international organizations of the World Health Organization (WHO) and Food and Agriculture of the United Nation (FAO) have recommended a number of requirements for probiotic agents to be used in food formulations. Of these, microbial species should be labelled and their strains should be specified if possible since probiotic-effect is well known to be strain-specific [4]. They have also recommended that probiotic bacteria should be counted precisely. Probiotic products should also be labelled with the viable bacterial count of the individual probiotic agent present at the end of the shelf life [5]. To obtain sufficient gut colonization, it has been suggested that probiotic bacteria should be administered at a daily concentration of $10^7$-$10^9$ colony-forming unit (CFU) [6]. Because of the GRAS status of the probiotics and the fact that probiotics are considered as food supplements rather than pharmaceutical agents, there are no or minimal standard regulations of their quality. However, poorly labelled products may indicate that its safety and efficiency cannot be assured. Inconsistencies between the labeled concentration and the actual count have been documented in a number of studies globally raising the need for the quality control of probiotic products used commercially [7-12]. Therefore, the aim of this study was to assess whether probiotic products available in the local market were correctly and sufficiently labeled with





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information regarding bacterial counts and nomenclature. Moreover, the study aimed at evaluating their *in vitro* antimicrobial activity as a potential of their health effect.

## 2. RESULTS

A number of eighteen probiotic products available in Iraqi community pharmacies were assessed in 2021-2022. Half of them (9) were used as oral capsules, 4 as oral drops, 2 as sachets and one product of each; vaginal tablet, oral tablet and oral suspension formulation were included (Table 1). All of these products are listed with their specific microorganism's content (Table 2); 11 out of 18 products (61.1%) contained single probiotic strain, 3 products (16.6%) had five probiotic strains in their formulation, 2 products (11.1%) listed four, one (5.5%) had three and one had two strains. Assessment also demonstrated that 9 products (50%) had their labelled organisms spelled and written correctly (genus and species nomenclature [15]; these were product-2, product-3, product-6, product-8, product-10, product-12, product-13, product-16 and product-17. Other products did not comply with the taxonomy and nomenclature of bacteria [15]. For instance, the genus of the bacterial species "acidophilus" was not mentioned in one product (product-5) while two other products stated the genus abbreviated with no annotation of the full name (product-9 and product-15). Although all genus were correctly written capitalized in all studied products, species were miswritten capitalized in six products where it should have been lowercased. Colony-forming unit has been used in the wright way as CFU (i.e. capital and in correct order) to express bacterial count in six products (product-11, product-10, product-8, product-3, product-2, product-15) while the term "kob" was used instead in product-1 oral capsules. Four other products also utilized the colony forming unit but not in the correct way (either lowercased or not in order). Three products used the expression live bacterial cultures, cells or spores to demonstrate the bacterial count in their formulations (Table 2). Product-14 package label had no unit to express the bacterial count while product-7 stated that 10mg of probiotic bacteria were formulated with no explanation of how this value corresponds to the actual bacterial count. Surprisingly, out the 18 tested products, only 7 (38.8%) products revealed positive growth when cultured on MRS and blood agar plates; these include product-1, product-4, product-5, product-11, product-12, product-15 and product-18. The calculated percentage of claim ranged between 0.0035 and 3700 with no product to match with the claimed bacterial count labelled on the package (Table 2) except for product-5 which had no specified probiotic count. It has also found that product-14 oral tablet and Lb plantarum was formulated tyndallised (not viable).

**Table 1.** Descriptive summary of the studied probiotic products.

| Total | Vaginal tablet | Oral capsule | Oral tablet | Sachet | Oral drops | Oral suspension |
|---|---|---|---|---|---|---|
| **18** | 1 | 9 (one gelatin) | 1 | 2 | 4 | 1 |

**Table 2.** List of the studied probiotic products with their labelled bacterial content and % of claim.

| Product label | Dosage form | Label organisms as listed in product package | Probiotics concentration as claimed in the product package | Cultured CFU/g | % of claim | Correct spelling |
|---|---|---|---|---|---|---|
| **1** | oral capsules | Saccharomyces Boulardii | $5x10^9$ kob/capsul (2cap=1g) | $1x10^7$ | 2 | No |
| **2** | gelatin capsules | Lactobacillus rhamnosus | 2 billion CFU/cap | 0 | NA | Yes |
| **3** | vaginal tablets | Lactobacillus acidophilus | 500 million CFU/tablet | 0 | NA | Yes |
| **4** | oral capsules | Lactobacillus Acidophilus | 3,6 MId UFC(3 cps) | $3x10^3$ | 0.1 | No |



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| | | | | | | |
|---|---|---|---|---|---|---|
| **5** | oral capsules | Acidophilus | Not stated | $2.7x10^6$ | NA | No |
| **6** | oral suspension | Lactobacillus sporogenes | 30 million spores | 0 | NA | Yes |
| **7** | oral capsules | Lactobacillus Acidophilus | 10mg | 0 | NA | No |
| **8** | oral capsules | Lactobacillus acidophilus | 10 billion CFU/g - 6mg/capsul | 0 | NA | Yes |
| **9** | oral capsules | L. acidophilus, L. rhamnosus, S. thermophilus, L. bulgaricus | 2 billion live cells/cap | 0 | NA | No |
| **10** | oral vials (water based) | Lactobacillus acidophilus LA 1688 Bacillus coagulans MTCC 5260 Bifidobacterium infantis ATCC 15702 Lactobacillus bulgaricus ATCC 11842-7995 Streptococcus termophilus FP 1622 | 6 BILLION/ CFU/single-dose vial | 0 | NA | Yes |
| **11** | oral drops (oil based) | Lactobacillus Rhamnosus, Lactobacillus Reuteri | 8 Billion CFU, 2 Billion CFU/ 1mL | $3.7x10^{10}$ | 3700 | No |
| **12** | oral drops (oil based) | Lactobacillus rhamnosus | $1x10^9$ lyophilised live bacterial cultures/1 drop | $5x10^8$ | 50 | Yes |
| **13** | oral drops (oil based) | Lactobacillus rhamnosus GG | $1x10^9$ cfu/ 5 drop | 0 | NA | Yes |
| **14** | oral tablets | Tyndallised Lb plantarum * (*; cells, non-viable, deactivated by means of heat treatment), Lactobacillus bulgaricus, Streptococcus thermophilus | 2 billion/ tablet | 0 | NA | No |
| **15** | sachets | L. helveticus, Bifidobacterium sp., L. acidophilus, L. bulgaricus, Str. thermophilus | $5x10^9$ CFU/ sachet | $2.5x10^6$ | 0.05 | No |
| **16** | oral capsules | Lactobacillus acidophilus | 1 billion cfu/cap | 0 | NA | Yes |
| **17** | oral capsules | Lactobacillus plantarum 299v (LP299V®), Lactobacillus plantarum LP90, Lactobacillus bulgaricus LB42, | 18 Billion Live Cultures/cap | 0 | NA | Yes |


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| | | | | | | |
|---|---|---|---|---|---|---|
| | | Lactobacillus paracasei LC86, Lactobacillus salivarius LS97 | | | | |
| **18** | sachets | Lactobacillus Casei, Lactobacillus Acidophilus, Streptococcus Thermophilus, Lactobacillus Rhamnosus | $1x10^9$, $1x10^9$, $1x10^9$, $1x10^9$ UFC/1 sachet (2.5g) | $1.4x10^4$ | 0.0035 | No |

NA: Not applicable

Out of the 7 products showing positive growth when cultured *in vitro*, only one 24 h-cultured product-supernatant (product-1) showed an inhibition zone on nutrient plates swabbed with *S. aureus* (Figure 1). The average inhibition zone diameter was (25 ± 2 mm). None of the probiotic-supernatants tested showed a zone of inhibition against *Escherecia coli* growth (Figure below).

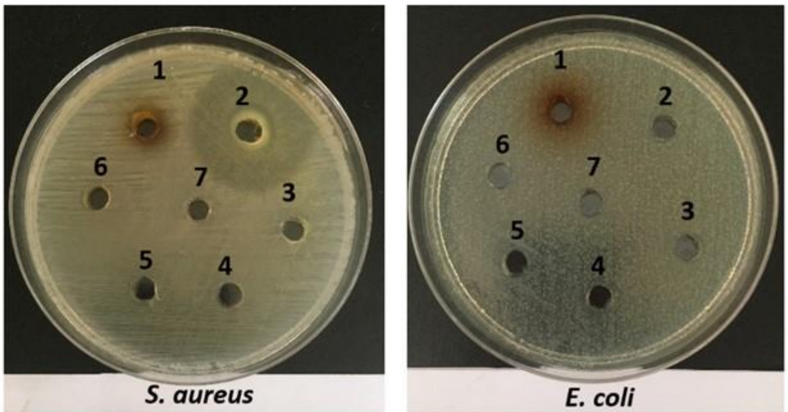


**Figure 1.** Antimicrobial activity of probiotic-supernatant against *S. aureus* and *E. coli*. A zone of no growth is noted around the well of *S. boulardii* supernatant indicating antimicrobial activity against *S. aureus*. 1; product-4, 2; product-1, 3; product-5, 4; product-15, 5; product-18, 6; product-12, 7; product-11.

## 3. DISCUSSION

Probiotics have been considered as an old-new pan-pharmacon due to their diverse prophylactic and therapeutic properties against many clinical situations [16-19]. The global market of these products has been raising especially after the pandemic of COVID-19 to attain an approximate value of USD 74.69 billion by the end of 2025 [20]. The same scenario should be applied to the Iraqi probiotics market. Though, there are no published related credential reports yet. However, in 2021, Ali and his colleagues published a piece of work affirming the deficiency in the regulation within the pharmaceutical sector of Iraq despite the efforts of the Iraqi government to provide adequate and safe medicinal products [21]. The published documentary highlighted the crucial need for the quality control of medicinal products including probiotics in the Iraqi market. Probiotic-beneficial effects have been documented to be strain-specific and influenced by the quantity of living cells approaching the target body sites [22]. Therefore, there is a demand for products to be labelled correctly for commercial use [7]. According to the Guidelines for the Evaluation of Probiotics in Food (2002), probiotic bacteria should be named in accordance with the international Code of nomenclature [23]. In addition to strain-specification, WHO and FAO also recommended that products should be labelled with the viable count of the individual strain at the end of the products shelf-life. In the current study, no product has complied completely with these requirements. However, half of the studied products stated the correct genus and species of the included probiotics. This was not surprising since many studies evaluating the quality of probiotic products in many areas of the world (Europe, America, Asia, Australia and South Africa) have shown comparable lack of accuracy findings [7-12]. For instance, many formulations did not state the actual


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names or that probiotics were misidentified with no strain specification. It has also been found that the total count was labelled to describe the concentration of more than one bacterial strain in many products tested. This goes in accordance with the current observation except for two products where the concentrations of the individual probiotic species were labelled. Spelling mistakes were encountered in 9 (50 %) products. This high percentage is analogous with the results observed in a study conducted on veterinary probiotic products [24]. These errors should not necessarily indicate poor quality products in terms of active ingredients but it undoubtedly highlights the lack of adequate manufacturer's knowledge and might negatively influence the consumer's trust in the quality of the products. In addition, most, if not all, of the published studies emphasized a finding of a mismatch between the labelled bacterial concentrations and the actual viable bacterial count of cultured cells. Similarly, the current study found that only 7 out of the total 18 products tested gave viable growth on culture media and that all of the counts mismatched with the stated concentrations labelled on the package. It is difficult to decide whether the resultant low viable bacterial count was encountered at the process of manufacturing or that the stated count was not contained at all. It might also be due to the inappropriate storage conditions of the products, taking into consideration the Iraqi hot climate in summer where temperature could reach > 40 ˚C. Testing the products before their expiry date suggests a further futuristic decline in the viable counts [7]. It has been recommended that storing probiotic products at 4 ˚C is perfect to maintain viability [7]. However, this statement was not designated on the leaflet of any of the products tested.

Of the liquid formulations, two of three oil-based oral drops were found to contain viable cells of high concentrations which might suggest a preserving property of the oil base [25], a conventional method used for preserving bacterial cultures [26]. One product (product-6) had both antibiotic and probiotic in the same powder formulation, this co-formulation may result in inhibitory effect of the antibiotic on the probiotic bacteria.

The definition of the WHO recommends that probiotics should be alive following administration to exert their beneficial effects [1]. However, we found that one of the studied products (product-14) did not match this definition as the probiotic was formulated in the tyndallised inactive form. This product might conversely influence health and should have been tested for immunogenicity [27].

Notably, no one of the studied products contain pathogenic bacteria known to cause infectious diseases such as enterococci [5]. Using such bacteria as probiotics is not recommended because of the risk of antibiotic resistance [28] and the possibility they transfer resistance virulence genes to other bacteria [29]. However, this study has serious limitations that identification was based on morphology and biochemical tests at the genus level. Molecular techniques may be more successful in identifying bacteria at the species or strain levels.

Although there has been no clear referral to a specific antimicrobial activity of the probiotic bacteria in any products, it was worth testing it in this study since antimicrobial effect is one of the utmost known health potentials of probiotics [30]. *Saccharomyces boulardii* (product-1) was the only isolate that showed inhibitory effect on *S. aureus*. In a study conducted by Venkateswarulu T.C and colleagues [31], extracted antimicrobial peptides of *S. boulardii* demonstrated antimicrobial activity against a number of potential pathogens including *S. aureus*. While product-1 is indicated for use to alleviate bowel disorders, no *in vitro* antimicrobial effect has been shown against the tested enteric coli. However, this does not mean the product is not effective, since probiotics can exert their beneficial effects via a variety of mechanisms other than antimicrobial activity [32]. For instance, *S. boulardii* was shown to have anti-toxin rather than cytolytic effect against *E. coli* [33]. The lack of *in vitro* inhibitory effect of the other 6 products' spent cultures may be due to the short incubation period of 24 h since some species might need a longer incubation period to synthesize their antimicrobial elements against the target pathogen or might have other health effective machinery [32].

## 4. CONCLUSION

In accordance with the previous studies conducted on commercial probiotic products, there is an urgent need for clear regulations and standard quality control operations of probiotic products by experienced organizations. Assessment of the influence of improper storage is also a very important demand.

## 5. MATERIALS AND METHODS

A total of 18 commercial probiotics containing products of different dosage forms were purchased as over the counter medicine from community pharmacies in Mosul/Iraq in the period between June/2021 and January/2022. None of the products has surpassed the expiry date during the running experiments and a


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minimum of one-and-a-half-year shelf life was encountered. The products' labels were veiled and the products were randomely coded with numbers from 1 to 18. All products were orally taken except one intended for vaginal use. The products were stored in the fridge at 4 ˚C for testing. Three media were used for culturing; blood agar, De Man Rogosa and Sharpe agar (MRS agar) and Sabouraud Dextrose Agar for yeast. One gram of the dry probiotic product or 1 mL of the liquid product was measured and dissolved in 9 mL sterile 0.9 % NaCl. Ten-fold dilutions were then made serially up to four dilutions. Twenty microliter volume of each dilution were then inoculated on two MRS (for lactobacilli isolation) and two blood agar plates (for other probiotic strains) in triplicate using Miles and Misra plate method [13]. One plate of each medium was then incubated either aerobically or anaerobically at 37 ˚C until colonies had grown sufficiently for visual counting (24-48 h). The obtained colony numbers were averaged, divided by the dilution factor and the inoculated volume to obtain the total count of the grown bacterial cells as colony forming unit per gram (CFU/g). Identification of bacteria was performed preliminary basing on the colony morphology and Gram staining. Some biochemical tests were also performed for further identification (catalase test was used for identification of lactic acid bacteria). In products were more than one *Lactobacillus* spp. are listed, no effort was paid to identify the different species because of the difficulty in specific counting. The percentage of claim was calculated according to the following formula:

$$\text{Actual count (CFU/g) / Label claim (CFU/g)} \times 100$$

To assess the antimicrobial effect of probiotic products, well-diffusion method was used [14] with minor modification. Briefly, a few colonies of each probiotic product that had been already grown on MRS agar plates (above) were allowed to grow in brain heart infusion broth for 24 h. At the end of the incubation period, each product's spent culture was centrifuged and the resulted supernatant was filtered to get a cell-free supernatant. Nutrient agar plates were prepared and swabbed with either *S. aureus* NCTC 6571 (as a reference of Gram-positive bacteria) or *Escherichia coli* NCTC 9001 (as a reference of Gram-negative bacteria). Seven wells were then punctured on each plate and 150 µL of each of the cell-free supernatants were pipetted into the corresponding well. Following incubation at 37 ˚C for 24 h, plates were inspected for the presence of zone of no growth around each well.

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