BACTERIAL CONTAMINATION IN MULTIVITAMIN SYRUPS

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ABSTRACT

Objective: To determine the bio-burden of isolated bacteria from multi-vitamin syrups manufactured by local industry of Peshawar.

Methodology: This cross-sectional study was conducted at Sarhad University of Information Technology and Khyber Medical University Peshawar, from July 2017 to December 2017. Microbiological quantification of nine different multivitamin syrups purchased randomly from different medicine stores was performed. The microbial load was determined by using the viable cell count method. Sample preparation was done through serial dilution and identification of bacteria was made on the basis of morphological and colonial characteristics, and by using biochemical tests. Antibiotic susceptibility profiles were determined by the Kirby Bauer disk diffusion method and interpretations were made according to Clinical and Laboratory Standards Institute (CLSI) guidelines. A total of 9 different antibiotics were tested against gram-positive bacteria while 7 different antibiotics were tested against gram-negative bacteria.

Results: All samples were found to be contaminated and the bio-burden exceeded the permissible limit set by the United States Pharmacopeia (USP). The overall frequency of Staphylococcus aureus was 55 % followed by Escherichia coli at 44%, Pseudomonas aeruginosa at 33% and Salmonella spp at 22%. All samples showed a pH within range of 5 to 9. Out of the gram-positive isolates, highest sensitivity (100%) was observed to Gentamycin, Ciprofloxacin, Chloramphenicol and Vancomycin. Pseudomonas aeruginosa was 100% sensitive to Ofloxacin, Gentamicin and Meropenem. Escherichia coli showed high sensitivity to Meropenem (100%) and Oflaxacin (100%) followed by Gentamicin (75%). Salmonella spp showed maximum sensitivity to Gentamicin (100%), Ofloxacin (100%) and Meropenem (100%). While high resistance was observed to Amoxicillin (100%), Cefotaxime (100%) and Clindamycin (50%).

Conclusion: All samples were contaminated with pathogenic bacteria. Most of the bacteria were sensitive to antibiotics.

Key Words: Aseptic technique, Bacterial contamination, Multivitamin syrup

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INTRODUCTION

Vitamins are a group of organic nutrients required in small quantities for a variety of biochemical functions that generally cannot be synthesized by the body, and must therefore be supplied in the diet[1]. Vitamin syrups are a non-sterile liquid dosage form mostly prepared for oral administration in children[2]. Different companies use various preservatives that have antimicrobial activity and these protect syrups from microbial contamination by inhibiting their growth[3]. An appropriate composition of preservatives is necessary. Otherwise there may be microbial contamination in the syrups[4]. Other factors such as proper handling and storage conditions also play important roles for preventing the growth of microorganisms[5].

Multivitamin syrups contain nutrient rich ingredients which act as substrate for the growth of microorganisms[6]. These microorganisms can deteriorate active ingredients and can interfere with the desired activity of

the product and may produce some metabolites that are toxic to the consumer[7,8]. Such toxic metabolites can cause illnesses including abdominal discomfort, diarrhea and acute gastroenteritis. Microbial contamination in syrups may also contribute to secondary bacterial infections in pediatric patients[9,11]. The presence of both pathogenic and non-pathogenic microorganisms in pharmaceutical products can cause various problems. Non-pathogenic microbes cause changes in the quality of syrups and affect the stability and position of the product[9]. Syrups must be prepared under aseptic conditions and the number and type of microorganisms must not exceed the acceptable level of < 102 cfu/ml according to the USP for non sterile syrups[12,13].

The most commonly reported microbial contamination found in syrups are Pseudomonas species, Salmonella spp. and some related gram-negative rods[14,15]. Escherichia spp., Klebsiella spp., Pseudomonas spp., Proteus spp., Enterococcus spp., Micrococcus spp., Salmonella spp., Staphylococcal spp. and Bacillus spp. have also been reported in syrups by a previous study[16].

OBJECTIVE:

The objective of this study was to determine the bacterial contamination in multivitamin syrups manufactured by the local industry of Peshawar in Pakistan.

METHODOLOGY

This cross-sectional study was carried out at Sarhad University of Information Technology and the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar from July 2017 to December 2017. Samples were collected randomly from different medicine stores of Peshawar. In this study, nine samples of multivitamin syrups, having manufacture and expiry dates along with batch numbers were analyzed. All of the samples were sealed and unused and coded as A, B, C, D, E, F, G, H and I. Samples were transported at room temperature to the microbiology laboratory of Sarhad University of Science and Information Technology and were analyzed for the presence of bacterial contamination. Sample preparation was done through serial dilution and pH was determined using pH paper.

Following serial dilution of the syrup, approximately 0.1 ml was taken from the 10^{-6} dilution, then it was spread on nutrient agar and incubated at 37 ^{o}C for 24 hours. After incubation, colonies were counted with the help of a colony counter. Pure culture was obtained for each colony followed by sub-culturing on nutrient agar. After sub-culturing, each pure culture was inoculated on different microbiological medias such as MacConkey agar, Eosin Methylene Blue agar, Mannitol Salt agar and Salmonella Shigella agar.

Identification of bacteria was made on the basis of morphological colonial characteristics, by using gram staining techniques and by using different biochemical tests such as catalase, oxidase, coagulase, indole, methyl red, urease and triple sugar iron. Antibiotic susceptibility tests were performed using the Kirby Bauer agar disk diffusion method in accordance with recommendations of the CLSI (2016).

RESULTS

Out of nine culture positive samples, the overall frequency of Staphylococcus aureus was five (55.5%), followed by Escherichia coli with four (44.4%), Psuedomonas aeruginosa with three (33.3%) and Salmonella spp. with two (22.2%) as shown in Table 1.

The pH of samples was determined to be within the range of 5 to 8. In all samples, the total viable count exceeded the standard limits set by the USP (10^2 cfu/ml) and by the BP (10^3 cfu/ml) as shown in table 2. The identity of isolated bacteria was confirmed by biochemical tests as shown in table 3.

The antibiotic susceptibility of isolated bacteria was studied and most of the bacteria were found sensitive to commonly used antibiotics as shown in figures 1 and 2.

DISCUSSION

In the current study, all multivitamin syrup samples collected from different medicine stores had a manufacturing date, expiry date, batch number and had new unused packs. All samples were found to be contaminated with pathogenic bacteria and bacterial colonies exceeded the standard limits set by the USP and the BP.

Possible causes of contamination and bacterial growth include improper handling, poor storage conditions, high contents of sugar and water and by not following standard protocols set by pharmacopeia. A number of factors such as nutrient composition, oxygen level and the pre- existing presence of microorganisms in the syrups can be responsible for spoilage of the product. In developing countries, the physical environment of pharmaceutical industries is not always adequate for maintaining high standards. They may have a poor water supply and lack facilities for hand washing; they may also have a deficiency of skilled workers and good pharmacy practices monitoring is not carried out regularly[17]. The four main bacterial species isolated from samples were Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Salmonella spp. All of these bacteria have the ability to cause human infections. The pH of all samples was found to be in the range of 5 to 9. This is in agreement with the findings of Emejuru et al[18].

Our values for the prevalence of Staphylococcus aureus (55%), Escherichia coli (44%), Salmonella spp. (22%) and Pseudomonas aeruginosa (33%) were similar to those from a study conducted by Mamun et al[19]. The small differences in isolated bacterial percentage may

Table 1: Frequency of bacteria in samples

Bacteria	Number of samples positive for bacteria	Percentage
Staphylococcus aureus	5	55.5%
Escherichia coli	4	33.3%
Psuedomonas aeruginosa	3	44.4%
Salmonella spp.	2	22.2%

Table 2: Determination of sample pH and the total viable count of isolated organisms

Multivitamin samples	pH	Total viable count (cfu/ml)	Organisms isolated
A	6.5	1.32×10^8	Staphylococcus aureus, Escherichia coli
B	5.0	1.43×10^9	Escherichia coli
C	6.0	1.23×10^8	Staphylococcus aureus
D	5.5	1.71×10^8	Staphylococcus aureus, Psuedomonas aeruginosa, Escherichia coli
E	6.0	1.53×10^9	Psuedomonas aeruginosa, Salmonella spp.
F	7.5	1.65×10^8	Psuedomonas aeruginosa, Salmonella spp.
G	7.0	1.20×10^7	Staphylococcus aureus
H	6.5	1.10×10^8	Staphylococcus aureus
I	5.0	1.26×10^7	Escherichia coli

Table 3: Biochemical identification of isolated bacteria

Bacteria	Biochemical tests									
	Catalase	Oxidase	Coagulase	Indole	MR test	Urease	TSI			
							Butt		H2S	Gas
Staphylococcus aureus	+	-	+	-	+	+	Y	Y	-	-
Psuedomonas aeruginosa	+	+	-	-	-	-	R	R	-	+
Escherichia coli	+	-	-	+	+	-	Y	Y	-	+
Salmonella spp.	+	-	-	-	+	-	Y	R	+	-

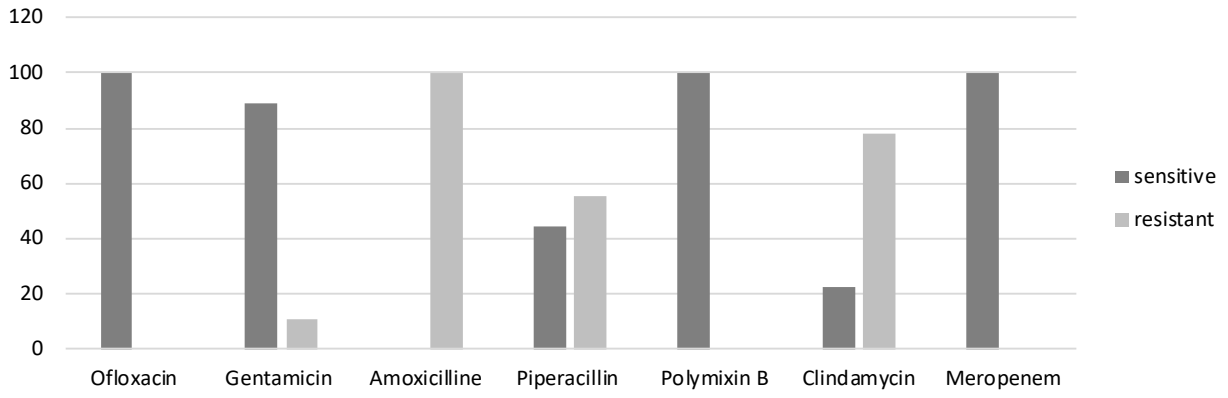

Figure 1: Overall antibiotic susceptibility against gram-negative isolates (part 1)

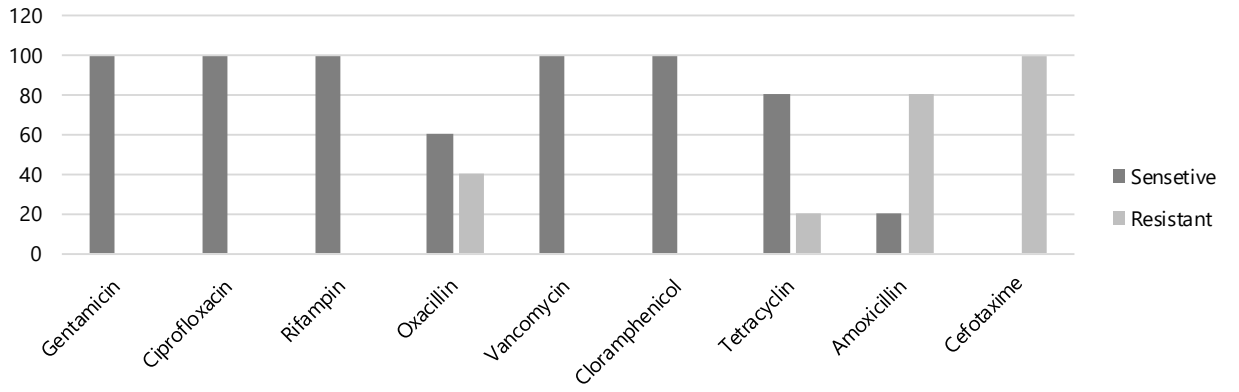

Figure 2: Overall antibiotic susceptibility against gram-negative isolates (part 2)

be due to differences in the sample size of both studies. Antibiotic susceptibility against the isolated Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa was determined and they were found to be resistant to Amoxicillin, Pipracillin and Clandamycin. High sensitivity was observed to Ciprofloxacin and Ofloxacin and our results are similar to those from studies conducted by Daniyan and Sandodere[16] and by Osungunna et al[20].

Despite the development in manufacturing techniques and hygiene practices, pharmaceutical products are still very much susceptible to microbial contamination if hygiene standards are not followed and the products are not stored properly. According to Hugo and Russell, the presence of microorganisms affects the quality of pharmaceutical products and the presence of 25% Pseudomonas aeruginosa is considered to be highly undesirable[21]. In the present study, Pseudomonas aeruginosa was at 33%, which indicates high contamination and poor hygiene conditions of local pharmaceutical industries in the region.

Similarly, various packaging is used for protecting pharmaceutical products and it is continuously changing with time to prevent contamination and increase shelf life of the products. In a study conducted by Delina et al., poor packaging caused all samples to be highly contaminated[22]. In the current study, some syrup samples were packaged in plastic bottles, which contain chemicals that can be released at high temperature. Such chemicals can contaminate syrups and may further affect the health of consumers.

CONCLUSION

This study concluded that all multivitamin syrup samples sold in Peshawar were highly contaminated with pathogenic bacteria. This study indicated that the local pharmaceutical industry in Peshawar do not follow standard protocols and aseptic techniques set by the USP and the BP for preparation of syrups and medicinal suspensions.

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CONTRIBUTORS

MA conceived the idea, wrote initial manuscript, collected data and carried out practical work related to this article. IU, AA and MI helped in refining the manuscript, data analysis and bibliography. MA, IA and YMY corrected language and grammatical mistakes, interpreted data according to objectives and revised the final manuscript. SZ and ZU helped in practical work, executing the project, data collection and literature review.