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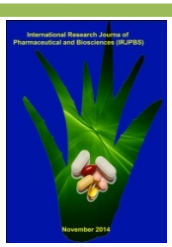

In-vitro Microbial Analyses of Extemporaneous Medications Prepared in A Tertiary Healthcare Facility in North East Nigeria

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Abstract

This study was designed to identify and isolate the microbial content of extemporaneous medications prepared in a tertiary health facility. Four different medications (Nevirapine, Hydrochlorothiazide, Furosemide and Captopril tablets) regularly formulated extemporaneously in the facility laboratory were investigated on the first, seventh and fourteenth day of each preparation. The storage conditions and mode of administration of the drug were mimicked within the duration of the study. Our findings established the presence of some microorganisms (*Staphylococcus aureus*, *Streptococcus* spp, *Aspergillus* spp, *Trychophyton* spp, *Microsporium* spp, *Mucor* spp and *Penicillium* spp), however, these contaminations were mostly within the official acceptable criteria for aqueous preparations used orally (i.e. not more than 10^2 and 10^1 CFU/ml or CFU/g for total aerobic microbial count and total combined yeast/mould count respectively). It can therefore be concluded that preparations from this health facility can be used without panic of inoculating pathogenic microorganisms to patients utilizing them.

Key words: Extemporaneous medications, Tertiary healthcare facility, Microbial, Analyses.

INTRODUCTION

Compounding is an integral part of pharmacy practice and is essential to the provision of health care [1]. Compounding according to the Pharmacopeia [1] refers to the preparation, mixing, assembling, altering, packaging and labelling of a drug, drug-delivery device, or device in accordance with a licensed practitioner's prescription, medication order, or initiative based on the practitioner–patient–pharmacist–compounder relationship in the course of professional practice.

The lack of commercially available oral liquid dosage forms is an ongoing problem for health care providers in many practice settings. Pharmacists are often challenged to provide extemporaneous oral liquids. While these are most commonly provided for paediatric patients where non-standard doses are more easily and accurately measured by using a liquid formulation, adults unable to swallow tablets or capsules and patients receiving medicines via nasogastric or gastrostomy tubes will also benefit from these preparations [2]. The world's population is ageing and many people suffer from dysphagia either as a consequence of disease or as part of the ageing process. Elderly patients are more prone to diseases linked to dysphagia, such as Parkinson's disease and Alzheimer's and other dementias, stroke and cancer [3-5].

A number of parameters need to be considered in the formulation of a stable oral liquid. These include chemical, physical, microbiological, therapeutic and toxicological stability evaluations [6]. There has been increasing number of reports of infections caused by contaminated non-sterile preparations [7]. The extent of microbial contamination depends on a number of factors such as availability of nutrients, presence of microorganisms and oxygen among others. Factors determining the outcome of medicament borne-infection include the type and degree of microbial contamination, route of administration and the state of the patient's immune system [8]. There is the need for Good Manufacturing Practice (GMP) in the production of non-sterile preparations like syrups and suspensions, which are specifically meant for children, to ensure that the products are consistently manufactured to a quality appropriate for its intended use [9]. This study is aimed at assessing the microbial stability of some extemporaneous preparations compounded in compounding laboratory of University of Maiduguri Teaching Hospital, Borno State, Nigeria.

MATERIALS AND METHODS

Sample preparation

A total of four (4) medications frequently compounded in the University of Maiduguri Teaching Hospital compounding laboratory were employed in the study; Nevirapine tablet 200 mg compounded to 50 mg/5 ml using vitamin B complex syrup as a vehicle, Tablet Hydrochlorothiazide 50 mg compounded to 10 mg/2.5 ml, Tablet Furosemide 40 mg compounded to 2 mg/2.5 ml and Tablet Captopril 25 mg compounded to 6.25 mg/2.5 ml.

Table 1: Extemporaneous preparations investigated

Samples	Constituents	Dosage forms	Mnf Date	Exp Date
A	Nevirapine	Suspension	05/02/15	18/02/15
B	Hydrochlorothiazide	Suspension	05/02/15	18/02/15
C	Furosemide	Suspension	05/02/15	18/02/15
D	Captopril	Suspension	05/02/15	18/02/15

Key: Mnf = Manufacturing, Exp = Expiring

Enumeration and Isolation of Microbial Contaminants

The extemporaneous medications to be used were agitated well prior to the withdrawal of 0.2 ml which was diluted serially (10 fold dilutions) in sterile normal saline. A quantity of 0.1 ml of the diluted sample was spread on the surface of Nutrient agar and Sabouraud's Dextrose agar (SDA) plates and cultured. Nutrient agar plate was incubated at 37 ^{o}C for 24 hr while the SDA plate was incubated at 25^{o}C for 3 days [10]. All experiments were done in triplicates and a control set up in each case. Colonies were counted and the mean numbers of colony forming units per ml of each were calculated [11]. These procedure was done on the first day (day 1), seventh day (day 7) and fourteenth day (day 14) respectively.

Identification of Isolated Microorganisms

The extemporaneous preparations were plated on various selective media such as MacConkey agar (for enteric bacteria), Blood agar (*Streptococcus*), Mannitol Salt agar (*Staphylococcus*) and Sabouraud's Dextrose Agar (mould and yeasts). Similar to [10], Growth characteristics, microscopy study and biochemical tests were used for the identification of isolates.

RESULTS

Table 2: Viable bacterial count in the extemporaneous preparations

Sample	Dosage form	Bacterial count (CFU/ml)		
		Day 1	Day 7	Day 14
A	Suspension	-	1.85×10^3	2.50×10^2
B	Suspension	-	1.95×10^3	2.00×10^2
C	Suspension	-	6.00×10^2	3.50×10^2
D	Suspension	-	1.90×10^3	1.00×10^2
Control		-	-	-

Key: - No growth, Control = sterile distilled water

Table 3: Distribution of bacterial contaminants in the compounded extemporaneous preparations on day one

Sample	Dosage form	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcal* specie
A	Suspension	-	-	-
B	Suspension	-	-	-
C	Suspension	-	-	-
D	Suspension	-	-	-
Control		-	-	-

Key: - absent, + present, Control = sterile distilled water

Table 4: Distribution of bacterial contaminants in the compounded extemporaneous preparations on day seven

Sample	Dosage form	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcal* specie
A	Suspension	-	+	+
B	Suspension	-	+	-
C	Suspension	-	+	-
D	Suspension	-	+	+
Control		-	-	-

Key: - absent, + present, Control = sterile distilled water

Table 5: Distribution of bacterial contaminants in the compounded extemporaneous preparations on the fourteenth day.

Sample	Dosage form	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcal* specie
A	Suspension	-	-	-
B	Suspension	-	+	+
C	Suspension	-	-	-
D	Suspension	-	+	+
Control		-	-	-

Key: - absent, + present, Control = sterile distilled water

Table 6: Distribution of mould/yeasts contaminants in the compounded extemporaneous preparations

Sample	Dosage form	Day 1	Day 7	Day 14
A	Suspension	*Aspergillus* spp	*Trychophyton* spp	*Penicillium* spp
B	Suspension	-	*Mucor* spp	*Microsporium* spp
C	Suspension	*Aspergillus* spp	*Aspergillus* spp	-
D	Suspension	*Aspergillus* spp	*Microsporium* spp	-
Control		-	-	-

Key: - absent, spp. = specie, Control = sterile distilled water

DISCUSSION

Our study revealed nil viable bacterial count on the first day of production of all the samples as shown in Table 2. On the contrary, variable levels of viable bacterial count were observed on the seventh and fourteenth day of production. On the seventh day, only one sample (Sample C) had viable bacterial count within the acceptable limit for non-sterile

pharmaceutical products based upon the total aerobic microbial count less than 10^3 CFU/g or CFU/ml [12]. All the four samples (A, B, C and D) had viable bacterial counts within the acceptable limit on the fourteenth day. The variable viable bacterial count in the samples studied could be as a result of either contamination of the distilled water used, media, personnel, containers or the inoculating environs [7].

Tables 3, 4 and 5 show the distribution of bacterial contaminants on the first, seventh and fourteenth day respectively. No viable bacteria were isolated on the first day of the sample production. These findings support the results presented in Table 2 for the first day of sample production. *Staphylococcus aureus* and *Streptococcal* spp were isolated in some of the samples on the seventh and fourteenth day which according to studies [7, 13-14] these are species associated with drug contamination. Coliform microorganism (e.g. *Escherichia coli*) which are mostly indications of faecal contaminants [15] was absent in all the samples on both the seventh and fourteenth day. These shows that the result is in concordance with several authors who advocates the absence of *Escherichia coli* in aqueous samples for oral use [10].

Yeast/moulds (*Aspergillus, Microsporium, Mucor, Penicillium* and *Trychophyton*) were found to be present on the first, seventh and fourteenth day of inoculation (Table 6). According to the European Pharmacopoeia, the limit for fungal/mould count in aqueous preparations for oral use is 10^1 CFU/g of CFU/ml. Our study was however narrowed as the count was not determined.

CONCLUSION

In conclusion, our study demonstrated that extemporaneously prepared medications from this facility can be used confidently without fear of inoculating pathogenic microorganisms to the patient since these contaminations are mostly within the official acceptable limit for aqueous preparations used orally (i.e. not more than 10^2 and 10^1 CFU/ml or CFU/g for total aerobic microbial count and total combined yeast/mould count respectively). However, the quality of distilled water, personnel, containers and formulation environs can still be improved upon considering the presence microbial growth found.

REFERRENCE

[1] Pharmaceutical Compounding Expert Committee. Pharmaceutical compounding. In: *United States Pharmacopeia* 34/National Formulary 29. Rockville, Md: United States Pharmacopeial Convention; 2011.

[2] Trissel, L. A. Trissel's Stability of Compounded Formulations. *American Pharmacists Association*. 4th ed.,Washington, DC: 2009.

[3] Kawamura, O., Easterling, C., Aslam, M., Rittmann, T., Hofmann, C. and Shaker, R. Laryngo-upper esophageal sphincter contractile reflex in humans deteriorates with age. *Gastroenterology* 2004: 127 (1):57-64.

[4] Morris, H. Administering drugs to patients with swallowing difficulties. *Nursing Times* 2005: 101(39):28-30.

[5] Ren, J., Xie, P., Lang, I. M., Bardan, E., Sui, Z. and Shaker, R. Deterioration of the pharyngo-UES contractile reflex in the elderly. *Laryngoscope* 2000: 110(9):1563-1566.

[6] Alison, H. and Beverley, D. G. Liquid Dosage Forms Extemporaneously Prepared from Commercially Available Products – Considering New Evidence on Stability. *J Pharm Pharm. Sci*. 2013: 16(3) 441 - 442.

[7] Ibezim, E. C., Esimone, C. O., Ofoefule, S. I. and Chah, K. F. Evaluation of the microbiological quality of some commercially available syrups and suspensions in Nigeria. *J. Phytomed. Therap*. 2002: 7(1&2): 18-25.

[8] Sykes, G. Pseudomonas aeruginosa contamination of liquid antacids: A Survey. *J. Pharm. Sci*. 1971:.60: 604-605.

[9] Coker, M. An assessment of microbial contamination during drug manufacturing in Ibadan, Nigeria, *Eur J Scientific Res*. 2005: 7: 19-23.

[10] Adeshina, A. O., Ajayi, S. and Onaolapo, J. A. Microbiological quality of some commercially available paediatric anti-malarial and cough preparations in Ilorin, Nigeria. *Nig. Journ. of Pharm Sci*. 2009: 8 (1): 109-117.

[11] Ibezim, E. C., Esimone, C. O., Ofoefule, S. I. and Chah, K. F. Evaluation of the microbiological quality of some commercially available syrups and suspensions in Nigeria. *J. Phytomed. Therap*. 2002: 7(1&2): 18-25.

[12] Maisonneuve, S. A. Microbial quality of pharmaceutical preparations. *European Pharmacopoeia.* 1975: p. 4451-4452.

[13] FIP Working Party. Microbiological purity of non-compulsory sterile pharmaceutical preparations. *Pharm. Acta. Helv*. 2nd report, 1975: 51: 33-40.

[14] Mendie, U.E. and Hugbo, P.G. The antibacterial potential of some intravenous fluid additives. *J. West African Pharm*. 1993: 7: 18-21.

[15] Sadia, K., Kamal, K. D., Sourav, B. and Rahed, N. Microbiological analysis of liquid oral drugs available in Bangladesh. 2013: 5 (4): 479-482.