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Full Length Research Paper

Microbiological quality of some brands of intravenous fluids produced in Nigeria

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Microbiological quality of some brands of intravenous fluids produced by some pharmaceutical companies in Nigeria was investigated. Membrane filtration method was used for concentration of contaminating organisms in the intravenous fluids. Thioglycollate medium, Tryptone Soya broth, Brilliant Green Agar, Pseudomonas medium, Mannitol salt agar, MacConkey agar, and Nutrient agar and Saboraud dextrose agar were the media used for the isolation and differentiation of the microbial species. Rabbitory method was used for Pyrogen test. Out of 160 samples analyzed, 14 (8.25%) were contaminated and remaining 146 (91.75%) were found sterile. The result of pyrogen test showed that 58 (36.25%) of the samples were pyrogenic. Dextrose (5%), peritoneal dialysis and Normal saline were free of detectable microbial species. Contaminating organisms in Dextrose Saline are *Micros*p*orum fulvum* and *Asp*e*rgillus* sp. In Half strength Darrow's *Bacillus cereus, Klebsiella pneumoniae* and *Asp*e*rgillus* sp. were detected. In Half strength Darrow's, Full strength Darrow's and Ringer Lactate, the contaminating bacterial species include *K. pneumoniae*, *M. fulvum*, *Asp*e*rgillus* sp. and *Penicillium* sp.

Key words: Intravenous fluids, sterile pharmaceuticals.

INTRODUCTION

Intravenous administration of fluids, drugs and nutrition is very common in hospitals (Waitt et al., 2004). In modern medical practice, up to 80% of hospitalized patients receive intravenous therapy at some points during their admission (Tjon and Ansani, 2000; Tager et al., 1983). These important life saving fluids have been reported as sources of life threa-tening infections and in some cases had been incrimi-nated as one of the strongest factors for morbidity and mortality associated with nosocomial infections in hospi-tals all over the world.

In United States, contaminated fluids was found to be the largest and most lethal known cause of outbreak of nosocomial infections, associated with wide spread distri-bution of contaminated medical product (Dennis et al., 1974). Between October, 1970 and March, 1971, eight United States Hospitals in seven states experienced 150 bacteremias caused by *Enterobacter cloacae*; there were nine deaths and all were associated with intravenous fluid therapy (CDC, 1997).

Nigeria is not an exception; there have been reported cases of circulation of contaminated intravenous fluids in hospitals in Nigeria. Some deaths and diseases condi-tions have been attributed to use of these microbiolo-gically unfit fluids. The Nigerian National Agency for Food, Drug Administration and Control (NAFDAC), have on many occasions ordered some intravenous fluids producing pharmaceutical companies to withdraw their products from market and stop further circulation of such products on account of microbiological defect. For instance, in a nation wide survey of intravenous products by NAFDAC in 2004, 566 samples comprised of 42 products from 8 manufacturers were tested for their microbiological fitness, 9 out of 42 products failed microbiological and pyrogen tests which are critical para-meters for the safety of such products. The products affected were Dextrose 5%, Dextrose 4.3% in 0.18%

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Table 1. Microbial contamination level of brand of intravenous fluids produced by some pharmaceutical companies in Nigeria.

Products	No. tested	No. contaminated (%)	No. sterile (%)
Normal saline	20	0 (0.0)	20 (100.0)
Dexrose 5%	20	0 (0.0)	20 (100.0)
Dexrose 5% + 0.9% NaCl	20	2 (10.0)	18 (90.0)
Dexrose 5% + 0.43% NaCl	20	4 (20.0)	16 (80.0)
Ringer Lactate	20	2 (10.0)	18 (90.0)
Half strength Darrow's	20	4 (20.0)	16 (80.0)
Full strength Darrow's	20	2 (10.0)	18 (90.0)
Peritoneal Dialysis solution	20	0 (0.0)	20 (100.0)
Total	160	14 (8.75)	146 (91.25)

NaCl, Darrow's half strength and Dextrose 50%. Immediate withdrawal of these declared contaminated products was ordered by NAFDAC.

Microbiological specification for intravenous fluids is that it must be sterile and pyrogen free throughout it shelf-life (European Pharmacopoeia, 2000). Reported cases of morbidity and mortality associated with use of intravenous are indications that these specifications are probably not met. The aim of this research therefore is to establish microbiological fitness of some of these intravenous fluids produced by some pharmaceutical companies in Nigeria.

MATERIALS AND METHODS

Media used and preparation

The media used for the microbiological analysis include, Nutrient Agar, Brilliant Green Agar, MacConkey Agar, Saboraude Dextrose Agar, Mannitol Salt agar, Tryptone Soya Broth and Thioglycollate Medium. The media were prepared according to the manufacturers instructions.

Sampling

Random samples of different brands of intravenous fluids produced by some notable pharmaceutical companies in Nigeria were purchased at different pharmaceutical stores in Ilorin and Zaria. 20 units of the following types of intravenous fluids were bought; 5% dextrose (500 ml), 5% dextrose in 0.9% NaCl (dextrose saline, 500 ml), 5% dextrose in 0.43% NaCl (500 ml), Half strength Darrow's (500 ml), Full strength Darrow's (500 ml), Peritoneal Dialysis (1000 ml), Saline (0.9 NaCl, 500 ml) and Ringer Lactate (500 ml).

Microbiological analysis

Membrane filtration method was used for the analysis. Two hundred milliliters (200 ml) was aseptically withdrawn from each unit of the samples, and filtered through a 0.45 µm (pore size) Millipore filter membrane. The membrane was aseptically removed from the filtration unit by using sterile forceps, cut into two parts and dropped the first part in Thioglycollate broth and the second part in Tryptone soya broth. The thioglycollate broth was incubated at 37^{o}C while the Tryptone soya broth was incubated at room temperature for seven days. The set up was observed on daily bases for visible turbidity that indicate microbial growth.

Any one with visible growth was removed and subculture into solid media for differentiation and characterization. All isolates were fully characterized biochemically.

Pyrogen test

The test was carried out by following procedures in British Pharmacopoeia (BP, 1993). Three rabbits which weighed not less than 1.5 kg each and already conditioned in the environment where the test was to be carried out, 2 days before the test, were used for each product. Before the injection of the product, Initial temperature of each rabbit was determined by taken the mean of two temperature difference and recorded. The sample was warmed to 38.5^{o}C before injection, and then appropriately diluted with pyrogen free isotonic sodium chloride solution. The product was then slowly injected into the marginal vein of the ear of each rabbit. The amount of sample injected varied; it depends on the type of product examined. The temperature of each injected rabbit was taken at interval of 30 min. The maximum temperature of each rabbit is the highest temperature recorded for the rabbit in 3 h after injection. The difference between the initial temperature and the maximum temperature of each rabbit is taken as its response. Where this difference was negative, the result was considered as zero response.

RESULTS AND DISSCUSION

A total of one-hundred and sixty (160) samples of intravenous fluids were analysed; fourteen (14) of these samples (8.75%) were microbiologically contaminated (Table 1) and one-hundred and fourty-six (146), representing 91.25% were sterile. Two bacterial and three fungal species isolated include *Bacillus cereus, Klebsiella pneumoniae*, *Cryptococcus fulvum*, *Aspergillus* spp. and *Penicillium* spp. (Table 2).

The result of pyrogen test showed that 58(36.25%) out of 160 samples analysed for pyrogen were positive, while 102 (63.75%) were negative for pyrogen (Table 3). Dexrose 5% + 0.43% NaCl was more pyrogenic, with 50% of the samples showed positive for pyrogen. Followed by Ringer Lactate and Full Strength Darrow's with 45% of the samples showing positive for pyrogen. The least pyrogenic product was Normal saline which gave

Table 2. Microbial contaminants isolated from brands of intravenous fluids produced by some pharmaceutical companies in Nigeria.

Microbial contaminants	Products							
	D5	DS	NS	HSD	FD	RL	PD	D5% + 0.43% NaCl
Bacillus cereus	-	-	-	+	+	+	-	-
Klebsiella pneumoniae	-	-	-	+	-	-	-	+
Micrococcus fulvum	-	+	-	-	+	+	-	+
Aspergillus spp.	-	+	-	+	+	-	-	+
Penicillium spp.	-	-	-	-	-	-	-	+

+ = Present; - = Absent; D5 = Dextrose 5%; DS = Dextrose Saline; NS = Normal Saline; HDS = Half Strength Darrow's; FSD = Full Strength Darrow's; RL = Ringer Lactate; PD = Peritoneal Dialysis.

Table 3. Pyrogenicity level of some brands of intravenous fluids produced by some pharmaceutical companies in Nigeria.

Products	No. tested	No. Pyrogenic (%) Response > 2.65	No. Apyrogen (%)
Normal saline	20	3 ((15.0)	17 (85.0)
Dexrose 5%	20	5 (25.0)	15 (75)
Dexrose 5% + 0.9% NaCl	20	7 (35.0)	13 (65)
Dexrose5% + 0.43% NaCl	20	10 (50.0)	10 (50.0)
Ringer Lactate	20	9 (45.0)	11 (55.0)
Half strength Darrow's	20	8 (40.0)	12 (60.0)
Full strength Darrow's	20	9 (45.0)	11 (55.0)
Peritoneal Dialysis solution	20	7 (35.0)	13 (65.0)
Total	160	58 (36.25)	102 (63.75)

pyrogenicity level of 15%.

Intravenous fluids are pharmaceutical products that are administered intravenously. Because of the rout of the administration, specification for any intravenous product is that, they must be sterile and pyrogen-fee. The need for sterility of the product arise because, presence of any living microorganism in the product meant to be passed into the blood stream could result in septicaemia and consequently blood stream infections. Several reports in the last decade have adduced cases of septicaemia to use of intravenous fluids (Twum-Danso et al., 1989; Ng et al., 1989; Bin-Ibrahim and Ghaznawi, 1990; Robert et al., 1990; Lacey and Want, 1991; Frean et al., 1994). Contamination level as high as 8.75% (Table 1) in products that suppose to be sterile is alarming and calls for overhauling of the production system by the pharmaceutical companies concerned. *K. pneumoniae*, *B. cereus* and *Aspergillus* sp. (Table 2) are nosocomial pathogens; their presence in the product could result to intrinsic nosocomial blood stream infection. Nosocomial blood stream infections have been found to a cause of death in United State, with mortality rate of 15% (Richard and Michael, 2001). Pyrogenicity level of 36.25% (Table 3) are rather too high; the reason for this may be attributed to contamination level of the products since, pyrogen is an endotoxin produced by some microorganisms especially gram-negative organisms. Infusion of pyrogenic product into already debilitated patients could only worsen the patients' condition and decrease their chances of survival.

From the above results it can be concluded that microbiological unfit products, supplied to the hospitals by the manufacturers, are significant factor that must not be over looked. Certificate of analysis of every batch of the product supplied must be demanded from the manufacturers to ensure that the batch has been properly controlled.

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