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EVALUATION OF THE EFFICACY OF TRANSFUSION FLUIDS PREPARED AT THE STERILE PREPARATION UNIT IN KENYATTA NATIONAL HOSPITAL.

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DEDICATION

I would like to dedicate this work to all those who have encouraged and helped me get this far.

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ABSTRACT

Transfusion fluids are parenteral solutions injected in relatively large volumes to restore the caloric or electrolytic balance of the body. They are introduced directly into the venous system or very rarely by hypodermoclysis. This, therefore, necessitates that they be sterile.

Sterilisation means an act or process of destroying completely all forms of microbial life including viruses. An article is either sterile or non-sterile and there is no such condition as partial sterility. It is, therefore, necessary to demonstrate that no viable organisms are present in the intravenous infusions. However, despite standards of quality control and general standards of good manufacturing practices, large scale epidemics caused by intrinsic contamination (that is, microbial contamination originating at the level of the manufacturer) of parenteral fluids have occurred. (Anon: Septicemias associated with contaminated intravenous fluids - Winconsin, Ohio, Morb Mortal Weekly Rep 22:99 March 1973)

The United States Pharmacopoeia Sterility tests are based on the concept that a negative result indicates absence of all micro-organisms and the testing methods are geared towards numbers of microbes rather than their identities. However, relationships between hosts and microbes vary. Many microbes thought to be commensal can cause infection, given the right circumstances, for example, normal intestinal flora. Therefore, until all conditions with regard to disease are known every organism is suspect (Dubois, RJ and Hirsch, J.9: Bacterial and Mycotic Infections of Man 4th Edition).

For these reasons it was the aim of this project to investigate both numbers and types of micro-organisms that my be found in intravenous transfusion fluids manufactured at the Sterile Preparation Unit at Kenyatta National Hospital and thereby test the sterility of the products before administration.

INTRODUCTION

Large volume parenterals are sterilised at the Sterile Preparation Unit (S.P.U.) by autocalving at 121°C for fifteen minutes as is officially specified in the British Pharmacopoeia (B.P.) 1973. The Pharmacist has to ensure that the product remains sterile from the time of sterilisation up to completion of the transfusion and in order to achieve this the following factors must be taken into consideration.

(a) Selection of raw materials and compounding or preparation of the material in such a way so that the microbial load is minimal.

(b) Selection of packaging that will not interfere with the sterilisation process and yet will maintain sterility after sterilisation.

(c) Application of an adequate sterilisation process that is compatible with materials and packaging being sterilized.

(d) Verification of sterilization.

(e) Proper storage of sterile goods.

(f) Delivering, opening and use of material with re-contamination.

Sources and Nature of Contamination

According to official monographs microbial contamination of packaged transfusion fluids may be caused by the following organisms:-

(i) Escherichia coli. This is a Gram negative bacillus commonly found in food, faeces and water. It is a common cause of gastroenteritis in infants and urinary tract infections and abscesses in both children and adults.

(ii) Staphylococcus aureaus. This is a Gram positive coccus which is aerobic. It is carried by many individuals in the nose, throat or skin and may cause suppurative lesions.

(iii) Pseudomonas aeruginosa. This is a Gram negative aerobic bacillus. It is resistant to many drugs and has simple nutritional requirements. If it multiplies in patient's wounds it can cause serious local and even systemic infections.

(iv) Fungi mainly Apergillus and candida albicans.

Contamination with these organisms could arise due to one or more of the following factors:-

(a) Inadequate sterilisation, that is sterilisation temperature is not reached or the sterilisation time is not completed.

(b) Poor fit between bottle lip and rubber plug thus water cooling on the bottle can spread by capillary movement between the bottle thread and screw cap and enter the bottle contents. The process is encouraged if the bottle contains a vaccum as a result of rubber seal failure during heating up.

(c) There is considerable pressure and heat during autoclaving, therefore, hair-line cracks can occur and microbes can gain entry to the contents during storage through these cracks.

(d) Contamination from raw materials such as distilled water, glucose, sodium chloride.

(e) Contamination arising from the operator, the furniture and fittings and the apparatus.

Assesment of microbial contamination

Viable micro-organisms from intravenous infusions subjected to a sterilisation procedure may occur in only a few items, be in extremely low numbers and in a damaged condition. Hence different media and longer incubation periods than used for standard microbial culture are required to encourage such organisms to replicate.

Items selected must be a representative of the whole batch and of sufficient number to give a satisfactory confidence to the prediction of contaminated items.

In the identification of bacteria certain criteria are followed.

(i) Morphology and Staining reactions of individual organisms generally serve as preliminary criteria, particularly for placing an unknown species in its appropriate biological group. A Gram-stained smear sufficies to show the Gram reaction, size, shape and grouping of the bacteria. Many different bacteria share similar morphological features and further tests must be applied, as below, to differentiate them.

(ii) Cultural characteristics including the growth requirements and appearance of culture to the naked eye are further criteria assisting identification. Attention is paid to size of colonies (on solid culture), their outline, their elevation, their translucency, whether they are colourless, white or otherwise pigmented and whether they produce any change in the medium (for example, haemolysis in blood-containing agar medium). However, simple observation of colonial morphology are insufficient for the differentiation of a species. Attempts are then made to culture organisms on media of different composition and incubated under a variety of conditions. The range of the conditions that support growth is characteristic of particular organisms. The ability or inability of the organism to grow on medium containing a selective inhibitory factor (for example, bile salt) may also be of diagnostic significance.

(iii) Species that cannot be distinguished by morphology and cultural characters may exhibit distinct differences in biochemical reactions and this is the other criteria used in the identification of bacteria.

There are numerous keys to bacterial identification and the best available at present for bacteria of medical interest is that of Cowan and Steel (1974). Their (first stage) approach is to determine the Gram reaction and then to observe the results of a limited series of morphological tests and biochemical reactions which enables one to assign the bacterium to a single genus or to two possible genera. For the second stage one refers to an appropriate table in which the individual species are identified primarily by a series of biochemical tests, involving such reactions as sugars fermentation, production of indole and hydrogen sulphide, the presence of urease, gelatinase etc.

When demonstrating contamination of a given fluid, certain controls must also be set up to validate the results obtained. The growth medium on to which inoculum is put must be sterile and its growth promoting properties must be demonstrated (B.P. 1973).

METHOD

i) Apparatus

The following apparatus were used in the experiment:-

(a) Autoclave for sterilising.
(b) Aseptic screen.
(c) Sterile growth media, for example nutrient agar, blood agar.
(d) Sterile petri dishes
(e) Sterile pasteur pipettes
(f) Sterile forceps
(g) Refrigerator
(h) Incubator.
(i) 500ml. bottles of intravenous fluids namely 5% W/V Dextrose and Normal Saline.
(j) Alcoholic cetrimide: 0.5% cetrimide in 70% Ethanol
(k) Pipette fillers.
(l) Wire loops
(m) Staining reagents
(n) Microscopic Slides
(o) Microscope
(p) Reagents and specific media to carry out biochemical tests.
(q) Other Standard laboratory equipment such as beakers, graduated measuring cylinders etc.

ii) Preparation of growth media

Growth media used were:-

a) Nutrient agar
b) Blood agar
c) Sabaraud's dextrose medium.

a) Nutrient agar was prepared by dissolving 28 grammes of nutrient agar powder in one litre of clean distilled water, with the aird of heat. It was then distributed into glass bottles, each carrying 100 millitres of the medium and autoclaved at 121 C for fifteen minutes. The nutrient agar so prepared was then used for the preparation of blood agar or for preparation of nutrient agar slopes used for sub-culturing or micro-organisms.

b) Blood agar was at every occasion prepared by melting 90 millilitres of pre-prepared nutrient agar (in a water bath) and then cooling to about 48 C. Ten millilitres of sterile defibrinated sheep blood was then added aseptically and the two were mixed gently. Approximately 20 millilitres of the medium was then poured into individual sterile petri dishes and the plates were allowed to dry thoroughly before inoculation. This was achieved by placing the plates in an oven at 37 C, which was previously swabbed with alcoholic cetrimide solution.

c) Sabaraud's dextrose medium was prepared by dissolving Sabaraud's dextrose dehydrate in water in the ratio of 30 grammes of powder in one litre of distilled water. Twenty millilitre portions of the medium were then transferred into 30 millilitre bottles, capped and then autoclaved at 121 C for fifteen minutes. The bottles were then stored in the refrigerator till they were to be used.

All other media were also stored in the refrigerator until they were required.

iii) Experimental Procedure

The procedure underlined here was followed in all subsequent experiments.

1. The following materials to be used in the experiment were thoroughly washed with detergent and rinsed several times with tap water and then distilled water. They were then wrapped in sterilising bags and autoclaved at 121°C for fifteen minutes:-
   - Pairs of forceps.
   - Pasteur pipettes
   - Petri dishes.

2. Samples from the Sterile Preparation Unit at Kenyatta National Hospital were then obtained.

   Each day six batches of each different type of infusion were made and each batch contained approximately two hundred bottles. Therefore, in order to obtain a practicable representative of each batch two bottles of 5% W/V Dextrose injection and two bottles of Normal Saline infusion were randomly selected from a batch each day so that in a week all six batches prepared at different time of the day were assessed. This is in compliance with the "Therapeutic Substances regulation" which specifies that the number of containers taken for test from every batch shall be two per cent of the containers in the batch.

3. The inside of an aseptic screen, including the working surface below it, were swabbed with alcoholic cetrimide solution and left for thirty minutes to allow dust and other particles to settle. Strictly, the inoculation process at all stages should be done in a laminar flow hood, thus minimizing chances of microbial contamination. This, however, was not possible and an aseptic screen was used. Thus contamination arising from various unsterile surfaces in the screen could arise. Allowance has, therefore, to be made for unavoidable contamination.

4. The following materials were also swabbed and thereafter kept under the aspetic screen.
   - Forceps still wrapped in sterilising bags.
   - Pasteur pipettes also wrapped sterilising bags.
   - Bottles containing appropriate media.
   - Petri dishes still wrapped in sterilising bags.
   - Bottles contain test fluids after removing the aluminium caps.
   - Pipette fillers.

5. The operator's hands were scrubbed with soap and brush up to the elbows and rinsed thoroughly. They were then wiped dry and swabbed with alcoholic cetrimide solution.

6. About twenty millilitres of blood agar was poured into the plates as described previously and after thorough drying the plate was divided into four using a water-proof marker on the underside.

   The pipette fillers were fixed on to the pipettes, care been taken not to allow the tip of the pipette to touch any surface. The rubber closures on test bottles were then removed using sterile forceps and approximately 0.2 millilitre aliquots of test fluid sucked and transferred on to the media in appropriate places as indicated below:-

(a) 5% W/V DEXTROSE	(b) 5% W/V DEXTROSE
(c) NORMAL SALINE	(d) NORMAL SALINE

The petri dishes were then covered and left under the screen for over an hour to ensure that the test fluid diffused into the medium. The medium was then incubated at 37°C for three days.

In a similar way 0.5 millitre aliquots of the test fluid were transferred into the Sabaraud's dextrose medium, the media bottles were closed by screw caps and incubated at 25°C (room temperature) for three days.

All plates were assigned a code number as were the media bottles.

7. If any growth occurred after three days then each different colony was subjected to certain tests (described below)and were also sub-cultured on to nutrient agar slopes by spreading a flamed wire-loop containing a part of the colony on the slope. The slopes were then incubated at 37°C for two days and stored in a refridgerator so that further tests could be carried out on the colonies later. Each bottle containing a colony on the nutrient agar slope was carefully marked with a code number.

If no growth occurred then the plates were incubated for a further five days. This was done to encourage any severely damaged organisms to recover and thus replicate. If still no growth occurred after this time then the plates were discarded and a conclusion drawn that the particular samples were sterile. The same procedure was followed for the Sabaraud's meduim.

For each batch of nutrient agar, blood agar and Sabaraud's medium prepared a series of control tests were done before inoculation of test sample. A plate of blood agar and nutrient agar were incubated at 37°C for three days, a bottle of Sabaraud's dextrose medium was incubated at 25°C for three days. This was to ensure that the media were not contaminated before inoculation. If any growth occurred in any of these then the media was discarded and a fresh batch prepared. Positive controls were also done to ensure that the media supported growth. For this purpose an exact duplicate of the test series was prepared, that is medium plus sample. Each medium was then inoculated with a few organisms appropriate to each particular medium. In both the blood agar and nutrient agar one millitre of a 1 in 100,000 dilution of an eighteen to twenty four hour fluid culture of Staphylococcus aureus was added, whilst in the Sabaraud's dextrose medium one millitre of a 1 in 1000 dilution of a twenty four to twenty eight hour fluid culture of Candida albicans was added. These were then incubated at 37°C and 25°C respectively, for five to seven days. All positive control tests had to be satisfactory with vigorous growth in all media inoculated with the organisms before the test was considered valid.

8. For all test samples the number of colonies growing on the plates were carefully counted using the surface count method. This is not very accurate but is useful for rough estimates of bacterial numbers. The shape and colour of the organisms were also noted. Any growth occurring in the Sabaraud's medium were also noted.

The following preliminary tests were then done on the colonies:

(a) Gram reaction - In this reaction a smear of the colony was made on a microscope slide with the aid of a flamed wire-loop and a drop of sterile normal saline solution. The smear was then allowed to dry and fixed by passing the slide through a Bunsen flame three times until the slide was just too hot to be comfortable when tested on the back of the hand. The slide was then allowed to cool and then stained with methyl violet (0.5% Methyl Violet 6B) for fifteen to thirty seconds. The stain was drained off and washed off with Iodine solution (prepared by dissolving one gramme Iodine in 100 millilitres of 2% potassium iodide solution). The slide was flooded with more Iodine solution and allowed to stand for thirty seconds. The iodine was then drained off and by holding the slide at an angle acetone-alcohol (equal volume of acetone and alcohol) was added drop by drop until the methyl violet ceased to stream out of the smear (about five to ten seconds). The slide was then immediately rinsed in water and stained with Neutral red (0.1% aqueous solution with one drop of 5% acetic acid added per one hundred millitres) for one to two minutes. The slide washed with water, blotted dry on a clean filter paper then examined with an oil immersion lens of the microscope.

If the cells were stained dark violet it was concluded that the micro-organisms were Gram positive whilst if they were pink they were Gram negative. The shapes of the micro-organism were also noted.

(b) Motility - A suspension of the organisms was placed on a glass slide, covered with a cover-slip and examined with the high-power dry objective of the microscope using a restricted amount of light. In this way it was possible to determine whether the organisms were motile.

(c) Catalase activity - In this test the organism was grown in nutrient broth after incubation over night and one millilitre of 3% hydrogen peroxide was added and the broth examined after five minutes. Evolution of gas indicated catalase activity.

(d) Oxidase (cytochrome oxidase) activity - Two to three drops of 1% tetramethyl -p- phenylenediamine dihydrochloride (prepared by dissolving one gramme of tetramethyl -p- phenylenediamine dihydrochloride in one hundred millilitres of distilled water and placed in an amber coloured bottle and stored in the refridgerator)was placed on a piece of filter paper and the test organism removed with a glass rod and smeared across the surface of the impregnated paper. A positive reaction was shown by the development of a dark purple colour within ten seconds. Positive and negative controls were done with pure cultures of Pseudomonas aeruginosa and Escherichia coli respectively.

(e) Acid from carbohydrates - one millilitre of phenol red solution was added to ninety millilites of peptone water and sterilised at 115° C for twenty minutes. One gramme of the appropriate sugar (glucose, lactose, sucrose or xylose) was dissolved in nine millilitres of water and steamed for thirty minutes. These were then added to the sterile base, distributed aseptically into sterile tubes with inverted Durham's tubes and steamed for thirty minutes.

These were then inoculated with culture and incubated at 37°C. The tubes were examined for seven days for acid or acid and gas production (gas production was indicated by presence of gas in the Durham's tube whilst acid production was indicated by a change in colour of the media from red to orange).

From the results obtained in these tests it was possible to draw a conclusion of the possible organism present in a particular test solution and further tests were then carried out to positively identify the organism. For example, if the results obtained from the preliminary tests were as follows:-

Morphology: Grey-white smooth colonies which haemolysed blood.
Gram reaction: Gram negative rods.
Motility: organism were motile.
Catalase: Showed a positive reaction.
Acid from carbohydrates: Acid and gas from sugars which indicates that organism attacked the sugars fermentatively and also produced acid from glucose.

From these results using the first-stage table for identification of medical bacteria it can be deduced that the organism present is an Enterobacteria thus the following reactions were carried out to determine the exact species of Enterobacteria.

(a) Growth in MacConkey's agar - The MacConkey's agar was prepared by weighing fifty two grammes of MacConkey's agar into a one litre round bottomed flask and one litre of freshly distilled water was added. The agar was then dissolved by heating with constant stirring. This was left to cool to about 50°C and dispensed in fifteen to twenty millilitre aliquots in screw-capped bottles which were then sterilised at 121°C for fifteen minutes.

The agar prepared when required was melted in a water bath and then poured aseptically into sterile plates. The plates were covered and left under the aseptic screen until the agar solidified. Using a flamed wire-loop the material to be investigated was streaked over the surface of the medium and the plates then covered and incubated at 37°C for two days.

MacConkey's medium contains bile salt, lactose and neutral red thus colonies of lactose-fermenting bacteria have a red colour on this medium due to acid production. Escherichia coli and Pseudomonas aeruginosa are two examples of bacteria forming acid on MacConkey's media. Salmonella, an enterobacteria is a non-lactose fermenter and will thus form colourless colonies.

(b) Methyl red reaction (M-R) - Escherichia coli and other enterobacteria have the ability to produce acid from glucose until the pH of the medium is lowered to 4.5 or less. This is the methyl red test and was another test used for the identification of the organisms obtained. In this test glucose phosphate broth was prepared in the way. 0.5 grammes of peptone and 0.5 grammes of potassium hydrogen phosphate were weighed and placed in a round bottom flask with one hundred millilitres of distilled water. This was steamed until all the solids dissolved. It was then filtered and the pH adjusted to 7.5 using a pH meter. 0.5 grammes of glucose was added and thoroughly mixed with the base medium. 1.5 millilitres of the medium was then distributed into tubes and these sterilised by autoclaving at 115°C for ten minutes.

The glucose phosphate medium was inoculated and incubated at 37°C for two days. After this time two drops of methyl red solution was added into each tube, the tube shaken and examined. A red colour indicated a positive reaction whilst a yellow colour indicated negative. A positive control was done with a pure culture of Escherichia coli.

After reading the methyl red reaction the same cultures were used for the Voges - Proskauer reaction.

(c) Voges - Proskauer reaction (V-P) - Some enterobacteria form acetyl methylcarbinol when growing in glucose phosphate broth and this forms the basis for the V-P reaction.

After completion of the methyl red test 0.6 millilitres of 5% alpha-naphthol solution and 0.2 millilitres 40% aqueous solution of potassium hydroxide was added into each tube, the tubes were shaken and then left to slope (to increase the size of the air/liquid interface). The tubes were examined after fifteen minutes and one hour. A positive reaction was indicated by a strong red colour. The genus Escherichia coli shows a negative reaction.

(d) Indole production - Enterobacterial strains produce indole when growing in peptone water or nutrient broth.

Nutrient broth was inoculated and incubated for forty eight hours. 0.5 millilitres of Kovac's reagent (hot saturated oxalic acid) was added and the medium shaken. This was examined after about one minute. A red colour in the reagent layer indicates indole. A positive control using a pure culture of Escherichia coli was also done.

(e) Potassium cyanide (KCN) test - Escherichia coli does not grow in KCN broth whilst other enterobacteria do, for example Klebsiella aerogenes.

KCN broth was prepared by weighing out 0.3 grammes peptone, 0.5 grammes sodium chloride, 0.0225 grammes potassium hydrogen phosphate, 0.564 grammes sodium hydrogen phosphate and dissolving these in one hundred millilitres of freshly distilled water in a round bottomed flask. The medium was filtered and distributed in twenty millilitre volumes in screw capped containers and sterilised at 115°C for twenty minutes. When required for use 0.3 millilitre of freshly prepared 0.5% KCN solution in sterile water was added to the twenty millilitre base. This was mixed and aseptically distributed in one millilitre amounts into sterile five millilitre screw-capped bottles. The medium was stored at 4°C.

The KCN base was inoculated with one loopful of an overnight broth culture and incubated at 37°C for forty eight hours. It was examined after twenty four and forty eight hours for turbidity indicating growth which constituted a positive reaction.

From these tests a good indication of which enterobacteria was present was obtained. Other tests were done to identify any other type of Gram negative rod-shaped bacteria which occurred and since the genus Pseudomonas was suspected the following tests were included:-

(f) Gelatin Liquefaction - This was shown by a test in which the organism grew in a nutrient medium solidified by gelatin and was prepared as follows:- 0.4 grammes of 5% bacteriological gelatin was soaked in five millilitres of distilled water until it was thoroughly softened. This was then added to one hundred millilitres of melted nutrient agar and mixed throughly. The media was sterilised at 115° C for ten minutes and then distributed aseptically into sterile petri dishes.

The medium was inoculated and incubated overnight at 37° C. The plates were then flooded with saturated ammonium sulphate solution. Haloes appeared around the colonies of organisms producing gelatinase.

(g) Tween-80 hydrolysis - Tween-80 is the oleic acid ester of a polyoxyalkylene derivative of sorbitan. It can be included in a suitable nutrient medium and the test culture(s) streaked on the surface, after incubation at the optimal temperature for the organism(s) under test, the plate is examined for opaque haloes around the growth. The opacity, which indicates lipolytic activity, is due to crystal formation.

The Tween-80 media was prepared by dissolving,by steaming, ten grammes peptone, five grammes sodium chloride, 0.1 grammes calcium chloride and twenty grammes agar in one litre of distilled water. The resultant media was then adjusted to pH 7.4 using a pH meter. Volumes of five hundred millilitres were sterilised at 115° C for thirty minutes in flasks and then cooled to between 40 and 50° C.

Ten millilitres of Tween-80 was sterilised at 121°C for fifteen minutes and five millilitres was added to each flask. A stroke inoculation was then made on the surface of the agar and incubated at 37°C. The plate was examined each day. A positive control with a a pure culture of Pseudomonas aeruginosa was done.

From the preliminary tests few Gram positive cocci were obtained and the only suspect organism was Staphylococcus aureus (conclusion drawn from results of initial tests) thus to show that these cocci were indeed Staphylococcus aureus a coagulase test was done.

In this test it was necessary to sub-culture the organisms into nutrient broth which was incubated at 37°C for twenty four hours, then to 0.5 millilitre of 1/10 dilution of plasma in saline, 0.1 millilitres of the broth culture of the organism was added. This was incubated in a water bath of 37°C and examined after one, three and six hours for a coagulum. Any negative tubes were left at room temperature overnight and then re-examined. A positive control using a pure culture of Staphylococcus aureus and a negative control of Escherichia coli were also set up.

Since only a few different organisms were found to grow from the intravenous fluids their identification did not prove to difficult and after only a few preliminary tests it was possible to assign the bacteria to their particular genus and thus further characterization tests were unnecessary.

A total number of forty samples were taken for testing which included twenty samples of 5% W/V Dextrose injection and twenty samples of Normal Saline infusion.

The results obtained were collected and tabulated.

RESULTS

No growth was noted in any of the Sabaraud's dextrose medium indicating absence of fungi.

a) Morphological features

5% W/V Dextrose injection.

Sample	Morphological features
1	Grey-white opaque colony that haemolysed blood
2	Smooth grey-white colonies
3	Grey flat spreading colonies that haemolysed blood.
4	White concave colonies
5	Grey flat colonies that haemolysed blood.
6	Pale cream concave colonies
7	Grey flat spreading colonies that haemolysed blood.
8	NO GROWTH
9	Grey white concave colony
10	Cream coloured concave colonies
11	Grey flat spreading colonies that haemolysed blood.
12	NO GROWTH
13	NO GROWTH
14	NO GROWTH
15	NO GROWTH
16	White smooth colonies
17	NO GROWTH
18	NO GROWTH
19	White smooth concave colonies
20	Grey flat spreading colonies

Normal Saline

Sample	Morphological features
1	White concave colonies
2	Grey concave colonies
3	Cream colonies which haemolysed blood
4	Grey flat spreading colonies which haemolysed blood.
5	Grey colonies which haemolysed blood.
6	Yellow colonies
7	Grey colonies
8	Grey flat spreading colonies
9	Grey colonies
10	NO GROWTH
11	NO GROWTH
12	White concave colonies
13	Yellow smooth shiny colonies
14	Yellow smooth colonies
15	White smooth colonies
16	NO GROWTH
17	Grey colonies
18	NO GROWTH
19	NO GROWTH
20	NO GROWTH

(b) Biochemical Tests

5 % W/V Dextrose injection

Test	Sample									
	1	2	3	4	5	6	7	8	9	10
Number of colonies	9	11	17	6	10	2	6	-	16	12
Gram reaction	-R	-R	-R	-R	-R	-R	-R		-R	-R
Motility	+	+	+	+	+	+	+		+	+
Catalase test	+	+	+	+	+	+	+		+	+
Oxidase test	-	-	+	-	+	+	+		-	-
Carbohydrate										
Fermentation and Oxidation	F	F	O	F	O	O	O		F	F
Growth in MacConkey's	+	+	+	+	+	+	+		+	+
Tween 80 hydrolysis	-	-	+	-	+	+	+			
Gelatin liquefaction	-	-	+	-	+	+	+			
Indole test	+	+		+					+	+
M-R test	+	+		+					+	+
V-P reaction	-	-		-					-	-
Coagulase test										
Growth in KCN	-	-								
Conclusion	EC	EC	Ps	EC	Ps	Ps	Ps	-	EC	EC

KEY:

+ Positive reaction

- Negative reaction

Ps Pseudomonas aeriginasa

EC Escherichia coli

St Staphylococcus aureus

R Rods

C Cocci

5% W/V DEXTROSE

Test	Sample									
	11	12	13	14	15	16	17	18	19	20
Number of colonies	8	-	-	-	-	12	-	-	6	16
Gram reaction	-R					-R			-R	-R
Motility	+					+			+	+
Catalase test	+					+			+	+
Oxidase test	+					-			-	+
Carbohydrate										
Fermentation and Oxidation	O					F			F	O
Growth in MacConkey's	+					+			+	+
Tween 80 hydrolysis	+									+
Galatin Liquefaction	+									+
Indole test						+			+	
M-R test						+			+	
V-P reaction						-			-	
Coagulase test										
Growth in KCN	+					-			-	+
Conclusion	Ps	-	-	-	-	EC	-	-	EC	Ps

Normal Saline

Test	Sample									
	1	2	3	4	5	6	7	8	9	10
Number of colonies	5	22	12	16	27	17	18	17	11	-
Gram reaction	-R	-R	+C	-R	-R	+C	-R	-R	-R	
Motility	+	+	-	+	+	-	+	+	+	
Catalase test	+	+	+	+	+	+	+	+	+	
Oxidase test	-	-	-	+	+	-	-	+	-	
Carbohydrate										
Fermentation and Oxidation	F	F		O	O		F	O	F	
Growth in MacConkey's	+	+	-	+	+	-	+	+	+	
Tween 80 hydrolysis	-	-	-	+	+	-		+		
Gelatin Liquefaction			-	+	+	-		+		
Indole test	+	+					+		+	
M-R test	+	+					+		+	
V-P test	-	-					-		-	
Coagulase test			+			+				
Growth in KCN.	-	-	-	+	+		-	+	-	
Conclusion	EC	EC	St	Ps	Ps	St	EC	Ps	EC	-

Normal Saline

Test	Sample										
	11	12	13	14	15	16	17	18	19	20	
Number of colonies	-	9	12	14	6	-	4	-	-	-	
Gram reaction		-R	+C	+C,	+C		-R				
Motility		+	-	-	-		+				
Catalase test		+	+	+	+		+				
Oxidase test		-	-	-	-		-				
Carbohydrate											
Fermentation and Oxidation		F					F				
Growth in MacConkey's		+	-	-	-		+				
Tween 80 hydrolysis		-	-	-	-						
Gelatin Liquefaction			-	-	-						
Indole test		+					+				
M-R test		+					+				
V-P reaction		-					-				
Coagulase test			+	+	+						
Grwoth in KCN.		-					-				
Conclusion	-	EC	St	St	St	-	EC	-	-	-	

DISCUSSION

Since the intravenous infusion fluids were subjected to a sterilisation procedure, very few exacting organisms were found to be present and thus only a few biochemical tests were required to ascertain which organisms were present. In all cases the primary objective was to identify those organisms that are specified in the official monographs to be ones that usually cause microbial contamination in packaged transfusion fluids since these present the greatest danger of infection.

It was assumed that all colonies were "pure", that is, each colony consisted of a single organism. This assumption was made since on sub-culturing on nutrient agar slopes the majority of the daughter colonies produced were identical to the parent. This, therefore, limited the number of tests required to identify organisms.

Since economy of time and material is necessary in most tests, heavy inoculation was essential for quick results. Moreover eliminating tests were applied in that when a particular organism was suspected a certain test was carried out and if negative results were obtained that particular culture was discarded. For example, if Pseudomonas aeruginosa was suspected the culture was plated onto gelatin agar. If hydrolysis did not occur then the culture was discarded. This type of elimination of culture involves the risk of loss of a rarely encountered organism or one that may be an unrecognized pathogen; this is the penalty that all must pay who discard their primary plates before they complete their investigations. However, in the ivestigation elimination tests were applied because the latter two types of organisms are unlikely to occur in "sterilised" transfusion fluids.

As shown in the results the number of colonies obtained in each sample was noted. However, these numbers are not very accurate in that large errors are inevitable due to the fact that a viable count assumes that a visible colony will develope from each organism. Bacteria are, however, rarely separated entirely from their fellows and are often clumped together in large numbers particullarly if they are actively reproducing. A single colony may, therefore, develope from one organism or from hundreds or even thousands of organisms. Each colony developes from one visible unit. Bacteria are seldom distributed evenly throughout a sample and as only small samples are usually examined very large errors can be introduced. Thus the number of colonies as noted in the results were only used as a guideline to indicate whether or not the amount of growth from a single test substance was considered significant or not. If the number of colonies obtained were extremely low (one or two colonies only), contamination from outside sources could be suspected though not concluded since it is possible that such low degree of contamination can occur in fluids that have gone through a sterilisation process.

All the transfusions had a volume of five hundred millilitres each. The effect of volume of parenteral on degree of contamination could not be investigated and the only variables used were the different types of infusions namely 5% W/V Dextrose and Normal Saline, and the different batches prepared at different times of the day.

A total of forty bottles of intravenous infusion fluid had been investigated. No fungal growth was seen in any of the samples.

Of the twenty samples of 5% W/V Dextrose injection investigated thirteen samples showed growth whilst fourteen samples of Normal Saline infusion samples showed growth of micro-organisms.

Thus out of a total of forty samples, 67.5% showed growth of which 32.5% was seen in the 5% W/V Dextrose injection solution and 35% was seen in the Normal Saline solution indicating that the type of infusion was not of very great significance in promoting growth of micro-organisms.

There was no significance in the results obtained from the different batches. From every different batch prepared at different times of the day at least two samples showed no growth and no single batch showed a significantly higher incidence of growth although it must be noted that out of six samples obtained from the sixth batch only three showed microbial growth, whilst in all other cases at least four or more samples showed growth. This may be due to the fact that whenever the sixth batch was about to be prepared fresh distilled water was required since by this time no distilled water was left. Any remaining distilled water was then left to stand overnight and used to prepare the other batches the next day. Although this was a likely source of contamination, this fact was not ascertained in the investigation.

Of the forty samples investigated the percentage of different organism isolated were as follows:-

Escherichia coli	32.5%
Pseudomonas aeruginosa	25%
Staphylococcus aureus	10%

It was noted that the organism staphylococcus aureus was only found to grow in the Normal Saline infusion.

CONCLUSION

From the results obtained in this investigation, it can be concluded that more than half of the transfusion fluids given in the hospital are not sterile before administration. The source of contamination was not, however, ascertained in the investigation. Nevertheless, common sources of contamination have already beed described in the introductory part of the project.

The commonest contaminant was found to be Escherichia coli, this strain of bacteria is commonly found in the intestine of man and animals and thus water is easily contaminated by it through the faeces and it is likely that water is the source of contamination, of the intravenous infusion fluids, by Escherichia coli.

Pseudomonas aeruginosa was also found in the samples. This species, commonly present in the human intestine, has the ability to grow in almost any moist situation over a wide temperature, needing only oxygen and a modest supply of nutrients. Consequently it can multiply in such preparations as intravenous infusion fluids quite readily.

A small percentage of Staphylococcus aureus was shown to be growing in a few samples of Normal Saline infusion. This organism is widely distributed, notably in air and dust and in human clothing. It is found as a commensal on the anterior nasal mucosa of 40 to 50% of healthy adults, in the throats of many of them, in the faeces of about 2% and on the skins of 5 to 10%. Thus contamination arising from this micro-organism is likely to come about due to the operator.

Normally routine tests for sterility are done to assess whether or not a particular batch should be rejected or not. These tests, however, are limited in their scope. As normally conducted, they will not detect the presence of virus, exacting parasitic bacteria or majority of thermophillic or psychrophillic bacteria.

Organisms that have been shocked, for example, by sub-lethal heat treatment, have specialised recovery conditions and these the routine test may not be able to provide. Aged bacterial spores may have long germination periods and may not grow within the period of testing and as tests are only conducted on a fraction of the total batch, low degrees of contamination are missed. The test for sterility, therefore, only detects relatively gross contamination in the final product.

In this study the tests were made more sensitive so that the type of contamination could be evaluated and the methods used were such that damaged organisms were given environments and time to recover and multiply.

Although it is true that no more than one or two viable types of micro-organisms in small numbers were found in any of the solutions sampled, infection in an already sick patient could result from a single organism.

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