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Microbiological evaluation of daily use handkerchief

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Abstract
Continuous increase in the production, distribution, and most importantly usage of economically packaged handkerchief is of utmost importance to the health of the public. This study was carried out to determine the microbial quality of randomly selected handkerchief in Ozoro, Delta State Nigeria from different persons. These randomly selected handkerchiefs were analyzed microbiologically. Microbiological examination showed the presence of bacteria and fungi isolates. Three bacteria species were identified; Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. A fungal was isolated; Candida albican. The total bacteria heterotrophic plate count ranges from 1.0×10^3 to 8.0×10^3. Staphylococcus aureus (50.0%) has the highest percentage occurrence while Klebsiella pneumonia (12.5%) has the least percentage occurrence. Candida albican had a percentage occurrence of 100%. The presence of these microbes in the used handkerchief has been traced to poor hygiene practices by handlers. It is recommended that; Handkerchief should not be used more than a day and individuals with flu should have separate handkerchief or tissue paper for controlling the flow of the flu and another for wiping sweat off their face.

Keywords: Microbiological, handkerchief, daily, evaluation, use

Introduction
Each area of the body surface acquires a characteristic flora of organisms well adapted to growth at that specified environment. This resident indigenous micro biota tends to suppress the intruders by competition for space and food supply or by the production of metabolites that are antagonistic to the survival of the intruder. These intruders could the dislodged from their environment when sneezing, coughing, belching, or yawning. Handkerchiefs are often used for wiping face, closing of the mouth or nose during the expression of reflex activities thereby constituting an abode for microorganisms.

A newspaper article describing a previous Life cycle analysis (LCA) on handkerchief versus tissue use (Black burn, 2009) [3] found that handkerchiefs were environmentally superior, but the

study did not define a use based functional unit and also assumed an exceptionally long life of the handkerchief (520 washes) compared to previously published LCAs on textile products (50 washes) (Laursen *et al.*, 2007; Collins and Aumonier, 2002) [9, 5].

Nose blow frequency can vary considerably between periods of respiratory illness day to day sneezes. Luckily medical investigations on transmission and treatment of the common cold help shield light on the frequency of nose blows during sickness for a study on the frequency transmission of chino virus cold by aerosols; Dick *et al.*, (1987) [6] Counted the number of times 12 participants blew their nose in a 12 hour study period in 4 separate experiments.

During colds, given the high frequency of nose blowing, the handkerchief will be used more extensively. During respiratory illness, I have assumed that the average American will blow his nose 8 times per handkerchief, in proportion to their relative surface areas. Using these relationship 112 handkerchiefs is used during illness as part of the functional unit.

Handkerchiefs often used in wiping face, closing mouth and nose when responding to reflex action or activities, therefore to enumerate the use of handkerchief, it can be done by using microscopic cell count and viable cell count. Microbes on daily used handkerchief can be as a result of the sterile handkerchief gotten in contact with the skin during reflex or involuntary action like sweat, coughing, sneezing, in these process microorganisms are transferred in large numbers to the handkerchief.

Microbiological Evaluation of daily use handkerchief is a research which gives general knowledge of the amount of microbes and other related organisms which can be found on daily use handkerchief.

This present study tends to evaluate the microbiological quality of used handkerchief and also to isolate and identify specific microorganism (Bacteria and fungi) from used Handkerchief.

Material and method

Study area

The study was conducted in Ozoro. Ozoro is in Isoko North L.G.A Delta State, the people are Isoko speaking and hospitable. Their main activities are food crop, farming accompanied by some hunting. They are also engaged in trade of food crop for cash to meet the other basic house hold needs. The region experience higher rainfall and humidity most of the year.

Collection of samples

A total of eight handkerchief eight different people were randomly selected. Samples were properly labeled and transported to the laboratory where their level of contamination and safety for human was determined.

Isolation of test organisms

The samples were swabbed using sterile swab stick and serially diluted according to Cheesbrough (2005) [4]. 0.5ml of 10^{-3}, 10^{-4}, and 10^{-5} dilutions were transferred to plates of nutrient agar. The media was prepared according to the manufacturer instruction and then used for enumeration of isolated bacteria.

The plates were incubated at 37^0C for 24 hours. Purified isolates where identified according to their morphological characteristics and reactions to biochemical test.

For fungal isolates, 1ml of 10^3, dilution was transfer to plates of Saboroud dextrose agar (SDA). Media prepared was according to the manufacturer instruction and then used for enumeration of isolated fungi. The plates were incubated at 23°C for 72 hours. The fungi were identified based on colonial/morphological characteristic.

Biochemical test

Citrate test

Citrate utilization test: using a sterile wire loop. A colony from purified sub cultures was isolated and stabbed straight down into the slanted agar medium.

The wire loop was removed, flame sterilized, and the inoculums was streaked on the surface of the stand. The test tube was covered tightly with a screw cap and labeled according for 24hrs at 37°C. It was then removed and observed for fermentation shown by a change in color from green to blue.

Oxidase test

Principle: A piece of filter paper is soaked with few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. If the test organism is oxidase producing, Kovac's oxidase reagent will be oxidized to a deep purple color.

- **Method:** Place a piece of filter paper in a clean Petri-dish and add 2 to 3 drops of freshly prepared oxidase reagent. Remove a colony of the test organism with a glass rod and smear it on the filter paper. Observe for development of a blue-purple color within 10 seconds.
- **Results:** Blue-purple color- oxidase produced. No blue - purple color -No oxidase produced

Indole test

Using a sterile wire loop a colony from the purified sub-cultures was isolated and inoculated into bijou bottle was then flamed. Sterilized and covered tightly with a screw cap and labeled accordingly, it was incubated for 24hrs at 37°C. The bijou bottles were then removed and 0.5ml of kovac's reagent was added. The bijou bottles were then shaken gently and left standing for 10mm. Examination for positive result was done by the formation of red color in form of a ring on the surface layer of the culture media.

Motility test

Using a sterile needle, the nutrient agar medium was inoculated to make 5 stables of the test organisms to the depth of 1-2cm of the bottom of the tube. Then test tubes were then incubated at 37°C for 24hrs. The line of incubation was examined for cloudiness showing the organisms are motile.

Results and discussion

Results

Table 1: Morphological and biological test on bacteria isolates.

Isolates	Gram stain	Morphological characteristic	Citrate	Catalase	Indole	Glucose	Lactose	H_2S	Gas
Staphylococcus aureus	GP	Cocci	-	+	+	+	+	-	+
Escherichia Coli	GN	Rods	-	+	+	+	+	-	+
Klebsiella pneumonia	GP	Rods	+	+	+	+	-	-	+

Key + = positive, - = Negative, GP = Gram Positive, GN = Gram Negative

Table 2: Shows the total plate count of Bacteria

Samples	Bacteria counts
Francesca I	$2.0x10^3$
Gloria I	$2.6 x10^3$
Priestly I	$1.8 x10^3$
Bernard I	$2.8 x10^3$
Francesca Il	$8.0 x10^3$
Gloria II	$1.0 x10^3$
Priestly II	$4.0 x10^3$
Bernard II	$1.5 x10^3$

Table 3: The percentage occurrence of bacteria isolates

Bacteria isolates	Percentage of occurrence
Staphylococcus aureus	50.0%
Escherichia coli	37.5%
Klebsiella pneumonia	12.5%
Total	100%

Table 4: The characteristics fungi isolates

Characteristics	Isolated fungi
Cultural characteristics	Creamy without profound growth
Colour of isolate	Creamy
Hyphae	No septate
Condidiospore	No septate upright
Conidia	Absent One cell globase in dry basipetal chain
Stolon	Absent
Rhizoid	Absent
Spore colour	Small glossy convex and smooth
Spore attachment Organism	Yeast pseudohyphae *Candida albican*

Table 5: The Percentage Occurrence of fungi isolate

Isolate	% of occurrence
Candida albican	100%

Discussion

The result of the study shows the presence of bacteria and fungi on handkerchief samples studied. Daily use handkerchief must be free from harmful micro organism that can cause serious ill health. *Staphylococcus aureus* (table 1) is a Gram positive, forms cluster, non-motile, non-spore forming and a facultative anaerobe. This bacterium test positive for Coagulase, Catalase and forms yellow colonizes on agar. It is mostly found on the nasal passage and axillae. The bacterium causes food poison, toxic shock syndrome, skin lesion. It can also cause bacteraemia, pneumonia and/or osteomyelitis. They get into surfaces by a number of ways which includes poor hygiene and sanitary conditions (Prescott *et al.*, 2008).

The presence of *E.coIi* in (Table l) signified that the handkerchief were contaminated and therefore not safe for human use, *E.coIi* in handkerchief sample is an indication of recent facal contamination microbiological standard in hygiene are necessary for a healthy life. (Singh, *et al*., 2002) [12]. *E. coli*, a Gram-negative, rod-shaped bacterium is commonly found in the lower intestine of warm-blooded organisms (endotherms). *E. coli* cells are a major component of feces, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination (Feng *et al*., 2002, Thompson and Andrea 2007) [7, 14].

Staphylococcus aureus has percentage occurrence of 50%, *E. coli* has an occurrence of 37.5% while *Klebsiella spp.* has an occurrence of 12.5%. The *Klebsiella spp.* whose presence may be as a result from contamination on handkerchief of sweat and other facial uses has stolen that good microbiological quality handkerchief was noted to be doubtful. (Public Health England, 2013) [11].

Candida albican had a percentage occurrence of 100% in all samples. The occurrence of various numbers of fungal species in the different handkerchief samples indicates the status of the treatment rendered to the handkerchief during production (LSO, 2010) [8].

Microorganism causes allergy and disturbance for different persons (Thompson, *et al.*, 2001) [13]. The occurrence of these fungi may cause diverse effects on human health as they have the potentials of producing mycotoxins. The concentration of these substances may increase during the use of handkerchief due to increase in the population of the fungi species, in the house or environment. Hence, daily use of handkerchief without proper washing of the handkerchief may cause harm or ill health to human and allergy can occur in some persons. (Aiello, *et al.* 2013) [2].

Conclusion and recommendations

Conclusion

The microbiological analysis of daily used handkerchief in Ozoro revealed the presence of pathogenic organisms (bacteria and fungi species) in the samples which indicate risk involved in daily use of such products and therefore could be hazardous to human health. Based on the results obtained in this study, it could be concluded that daily use handkerchief are contaminated with bacteria and fungi. The presence of these microbes in the used handkerchief has been traced to poor hygiene practices by handlers.

Recommendations

It is hereby recommended that;

1. Handkerchiefs should be washed at intervals;
2. Handkerchief should not be used more than a day.
3. Individuals with flu should have separate handkerchief or tissue paper for controlling the flow of the flu and another for wiping sweat off their face.

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