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Isolation and characterization of bacteria from babies heat prickly powder purchased in Imo State University, Owerri

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Abstract

Aim: To isolate and Characterize Bacteria from Babies Heat Prickly powders purchased in Owerri, Imo State.

Method: A total of Ten (10) Baby heat prickly powder samples were randomly purchased from different market locations in Umuahia, Abia state. Isolation and identification of Bacteria were conducted using standard microbiological techniques. Bacteria were Characterized using standard microbiological and biochemical test.

Results: The isolated Bacteria include *Staphylococcus aureus*, *Bacillus* sp, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* had the highest percentage occurrence (40%) while other Bacteria had (20%) each with the total bacteria count (TBC) ranging from 4.3×10^4 CFU/g to 1.32×10^5 CFU/g.

Conclusion: High bacterial load was observed in the present study which showed there was contamination of most of the products in respect to the international Microbiological standards recommended limits for bacterial contamination in powder/Cosmetic products (1.0×10^3 CFU/g) for bacteria. This could be as a result of poor manufacturing practices, poor hygiene, contaminated raw materials or the susceptibility of the ingredients contained in the baby powders.

Keywords: Baby heat prickly powder; Bacteria count; Bacteria contamination; Cosmetic products; Isolation

1. Introduction

The description of cosmetic powders functions can vary from decorative to protective. Powder is a cosmetic product used by people to improve their looks, prevent prickly heat (Mirhosseini et al., 2011) also inhibit the growth of bacterial pathogen which may cause unpleasant odor and sometimes skin infections (Michael et al, 2011). Their functions could include beautification, reduction in appearances of wrinkles, smoothening the skin, reduction of shininess caused by oily skin, prevention of prickly heat etc. Some powders with sunscreen can also reduce skin damage from harsh sunlight and environmental stress (Tran and Hitchins, 1994). Babies Heat prickly powders are powders that contains antibacterial, Absorbent and refreshing natural essential oil that aid in the relieving itching, heat rash and skin irritation on babies' skin from hot weather. This powder plays pivotal role in management of Miliaria or prickly heat (Rekha and Anoop, 2018) and also in Skin diseases, problems etc. Heat prickly powders can be made from Corn starch or Talc. Most baby powders are made of cornstarch with few additional soothing ingredients such as zinc, Chamomile extract, rose extract, kaolin and other soothing elements to soothe red and irritated skin. The talcum powder is another important ingredient in these baby powders because it has the ability of absorbing moisture, oils, odors and serving as lubricant although it produces astringent effect with the human skin. Talcum made powders when applied, talc particles become

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airborne and, when inhaled, can cause coughing, wheezing and, in severe cases may lead to chronic respiratory problems and death (Pairaudeau et al, 1991). Most of these Heat prickly powders could help in inhibition of bacteria growth (Huda and Saha, 2009) as some contain herbal actives such as Menthol (natural cooling agent), Neem, Ginger (Antimicrobial and provides instant relief) depending on the Active ingredient of the powder. These powders have been used in the reduction of one of the common disorders of the sweat glands in babies called Miliaria also known as Prickly heat which occurs due to high levels of heat and humidity. It is caused by obstruction of the sweat ducts, causing the eccrine sweat to leak into the epidermis (Williams and Grindlay, 2010). Miliaria is classified into 3 types according to the level of obstruction of the sweat duct (Champion et al, 1998). Miliaria crystallina, Miliaria Rubra, and Miliaria profunda (Wenzel and Horn, 1998). Excessive hydration of stratum corneum leads to transient blockage of sweat ducts because babies have immature eccrine sweat glands (Cui et al, 2017). The ability of microorganisms to grow and reproduce in cosmetic products has been known for many years (Fujital and Onyerad, 2005). These powders despite their functions provide a favorable condition for bacterial growth and also the versatile activities of Microorganism allow adaptation to a very broad range of environmental conditions. As a result, all classes of natural organic compounds are susceptible to degradation and synthetic compounds are also attacked. Contaminating microorganisms in cosmetic powder may cause a spoilage of the product and when pathogenic, they represent serious health risk for consumer's worldwide (Becks and Lorenzoni, 1995, and Behravan et al, 2005). Cosmetic powders such as baby powders, facial powders etc. could be contaminated either during their preparation (through raw material, ingredients and handling), storage, transportation and usage (Álvarez-Lerma et al., 2008). *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are the most common pathogenic bacteria found in cosmetic samples (Behravan et al, 2005). Many people are unaware that most baby powders could harbor bacteria despite their constituents. Others do not replace powders until it's completely finished despite how long they purchased and used them, the longer the microbes stay in powder, rapid growth and multiplication and production of metabolite would be expected, this could lead to biodegradation of product and hence the risk of infection to consumers and users of the product (Anderson and Parkin, 2007). Heat prickly powders has been on high demand for the reduction of rashes and other skin problems mostly in babies.

2. Material and methods

2.1. Sample collection

Samples were collected aseptically from Babies Heat prickly powders purchased in various market points located in Owerri and taken to the laboratory for microbiological analysis. A total of 10 samples were purchased which were strictly heat prickly powders for babies.

2.2. Media used and preparation

The media used for the microbiological analysis were prepared according to the manufacturer's instruction by dissolving the required amount of the media powder solution in a known volume of water and autoclaved at 121 °C for 15mins. The media used were Nutrient agar, Blood agar and Mannitol salt agar

2.3. Serial dilution

One gram (1 g) of each sample (Prickly heat powders) was weighed and dissolved in 9 ml of sterile distilled water in a Test-tube. Serial dilution of the powder sample and the digested slurry sample were carried out using Tenfold dilution method. Then the tenfold serial dilutions were performed, by which 1 ml from the sample was pipette into another test tube containing 9 ml of distilled water to give a 10¹ dilution factor and the sample was diluted serially up to 10⁶ aseptically and the test tubes were covered with cotton wool to avoid contamination.

2.4. Inoculation and isolation of bacteria

0.1 ml aliquot of the dilution were pipette aseptically into the surface of various sterile solid media plates which has been prepared such as Blood agar, Nutrient agar, Mannitol salt agar using spread plate method which a sterile glass rod was used to spread the Aliquot of the dilution over the surface of the various media respectively. The agar plates were incubated in an inverted position at 37 °C for 24 hours. At the end of the incubation period, the resulting colonies from the Agar plates were purified by sub-culturing on freshly prepared nutrient agar plates. The plates were incubated at 35 °C for 24hours. After overnight incubation, the resulting discrete colonies were stored in agar slant for further use.

2.5. Identification of the isolates

The identification of bacteria was based on morphological characteristics, Gram staining and biochemical tests carried out on the pure isolates. Morphological characteristics observed for the bacteria after 24 hours of growth included colony appearance, shape, elevation, edge, and pigmentation.

2.6. Gram Staining

Gram staining was performed according to the method as described by Cheesbrough (2010). This was carried out on discrete colonies. A smear from the pure culture was made on a grease free glass-slide by emulsifying in distilled water. The smear was heat fixed by passing over a flame. The prepared slide was flooded with crystal-violet solution for 1 minute, after which was rinsed off with water. *Lugol's iodine* was added for 1 minute and washed with distilled water. It was then decolorized briefly (30 seconds) by acetone and counterstained with safranin for about 2 minutes. The slides were again rinsed with distilled water and allowed to air dry in a safe cabinet free from dust and flies. A drop of immersion oil was added onto the Gram-stained smear and this was examined under the microscope using oil immersion objective lens (X100). Gram positive organisms appear blue/purple while Gram negative organisms appear pink/red (Cheesbrough, 2010).

2.7. Catalase Test

This is used to differentiate those bacteria that produce the enzyme catalase, such as *Staphylococcus* from non-catalase producing bacteria such as *Streptococcus*. A drop of hydrogen peroxide (H₂O₂) was placed on a grease free-slide and a 24 hours culture was then emulsified with the drop of H₂O₂ on the slide. Presence of bubbles was observed immediately as an indication for positive reaction.

2.8. Indole Test

The test shows the ability of certain bacteria to break down the amino acid tryptophan, in which one of the end products is indole which would be accumulated in the medium. This was detected using Kovac's reagent. The test organism was incubated at 37 °C for 48 hours, after which 0.5 ml of Kovac's reagent was added to each test tube and allow standing for two minutes. The positive reaction gives rise to red coloration at the top of the layer of the medium (Cheesbrough, 2010).

2.9. Methyl Red (MR) Test

This test determines the ability of microorganisms to oxidize glucose with the production and stabilization of high concentration of acid end products. A heavy inoculum of the test organism was inoculated into MR medium contained in each test tube. The tubes were then incubated for 48 hours at 37 °C. After that, 5 drops of methyl red indicator was added to the incubated test tubes. An instant red colour signifies a positive test (Cheesbrough, 2010).

2.10. Voges Proskauer (VP) test

It is used to determine the ability of many microorganisms to produce acetone (Acetyl methyl Carbinol) during fermentation of glucose. *Voges proskauer medium* in different test tubes were inoculated with heavy inoculum of the test organism. They were incubated at 37 °C for 48 hours. After incubation, 0.5 ml of alpha (α) naphthol was added, and then followed by 0.5 ml of 40% KOH (Potassium Hydroxide). It was then agitated and allowed standing for 30 minutes; a red to pink colour signifies a positive test (Cheesbrough, 2010).

2.11. Oxidase test

The enzyme, Oxidase, will oxidize the redox dye such as *tetramethyl paraphenylene diamine dihydrochloride* to deep purple colour. This enzyme is produced by some aerobic bacteria as part of their respiratory oxidation mechanism. A strip of Whatman's filter paper was soaked in freshly prepared 1% solution of *tetra methyl-phenylene-diamine dihydrochloride*. After draining, the strip is laid in a petridish and moistened with distilled water. The colony to be tested is picked up with platinum loop & smeared over the moist area. If positive, deep blue purple appears within 5-10seconds. If negative, there is no colour change.

2.12. Coagulase test

A drop of normal saline was placed on two separate slides using a loop, a portion of the isolated colony was emulsified in each drop to make two thick suspensions. A drop of human plasma was added to one of the suspensions and mixed gently. A coagulase positive result was indicated by clumping of colonies together.

2.13. Citrate Utilization Test

This test is used to demonstrate the ability of a microorganism to utilize citrate as the sole source of carbon and as energy source for their growth and ammonium salt as a sole source of nitrogen. A heavy inoculum of the test organism was inoculated into a sterile *Simmon's citrate medium* with the aid of sterile wire loop. The inoculated Bijou bottles were incubated at 37 °C for 72 hours. A positive test was observed by a turbid and change of colour of the medium from light green to blue (Cheesbrough, 2010).

2.14. Sugar Fermentation Test

This test helps to identify the microorganisms that can ferment the carbohydrates (glucose, sucrose and lactose). A 24 hours old culture was stabbed into a sterile triple sugar iron agar (TSI) slant in a Bijou bottle and incubated at 37 °C for 24 hours. It was then observed for glucose, lactose, sucrose, gas production and motility. In a positive test for glucose, was indicated by redness at the bottom of the bottle, in lactose, the media appeared yellow, for motility in the line of stab of the medium would not be sharply define and the rest of the medium would be cloudy (Cheesbrough, 2010).

2.15. Motility Test

This test is useful in detecting motile and non-motile microorganisms. A sterile straight wire was used to stab inoculate the Tube containing the agar with the test organism. The tubes were incubated for 24 hours. A diffuse hazy growth that spreads through the medium making it look opaque indicates a positive result, while non-motile bacteria had growth confined to the stab line with definite margin spreading to surrounding area.

2.16. Antibiotic susceptibility testing of the isolates

The standard Kirby-Bauer disk diffusion method was used to determine the antibacterial susceptibility profiles of the isolates. Pure isolates were cultured for antibiotic susceptibility assessment using the disc diffusion method (Obi, Bassey and Momba, 2004). The pure culture from suspensions were adjusted by using 0.5 McFarland standards and inoculated aseptically on Nutrient agar. Paper discs impregnated with antibiotics were placed on Nutrient Agar and incubated at 37°C for 24 hrs. Zones of inhibition were observed and measured after 24 hours. The commercial antibiotics used for the sensitivity profile include septrin (SXY), ciprofloxacin (CPX), Ampiclox (APX), Gentamycin (CN), amoxil (AMX), streptomycin (S), erythromycin (E), chloromphenicol (CH), augmentin (AU), and ofloxacin (OFX).

3. Results

Table 1 The cultural and morphological characteristic of bacterial strains

S/N	Colony Morphology	Microscopy	Suspected organism
1.	Golden yellow, circular, raised, smooth and opaque on MSA	Gram positive, cocci in grape-like clusters	<i>Staphylococcus aureus</i>
2.	White, circular, raised, smooth and opaque on B/A.	Gram positive, cocci in pairs	<i>Staphylococcus epidermidis</i>
3.	Green, circular, flat, smooth and opaque on N/A.	Gram negative, rods	<i>Pseudomonas aeruginosa</i>
4.	White, circular with jagged edge, flat, Dry and opaque on B/A.	Gram positive rods	<i>Bacillus sp.</i>

Keys: MSA= Mannitol salt agar, B/A= Blood agar, N/A= Nutrient agar, S/N= Serial number.

Table 1 shows the cultural, morphological of bacterial strains which were isolated from Babies heat prickly powder purchased in Owerri, Imo State. The bacteria isolated from the Babies heat prickly powder samples showed various pigments such as golden yellow, white and greenish colours according to the media used. Under microscopy it was identified that over 80% of bacteria isolated were gram positive because they were able to retain the crystal violet stain in their cell walls while 20% of the bacteria were gram negative because they were unable to retain the crystal violet stain in their cell wall.

Table 2 shows several biochemical tests such as Citrate utilization, Voges-Proskauer (VP) test, Methyl Red (MR) test, Indole production, Oxidase, Catalase, and Coagulase test which were carried out to identify these bacteria isolates

Table 2 The biochemical characteristic of bacterial strains

S/N	Ca	Co	Ind	MR	VP	Cit	Ox	Mot	Glu	Lac	Presumed organism
	+	+	-	+	+	-	+	-	+	+	<i>Staphylococcus aureus</i>
	+	-	-	-	+	-	-	-	+	+	<i>Staphylococcus epidermidis</i>
	+	-	-	-	-	+	+	+	+	-	<i>Pseudomonas aeruginosa</i>
	+	-	-	+	+	+	+	-	+	-	<i>Bacillus sp</i>

Key: Ca=Catalase, Co= Coagulase, Ind=Indole, MR=Methyl Red, VP= Voges-Proskaver, Cit= Citrate, Ox= oxidase, Mot=Motility, Glu=Glucose, Lac=Lactose, + = Positive; - = Negative, S/N=Serial number

Table 3 shows the distribution and the total bacteria count of Babies heat prickly powder samples. The total bacteria count for the samples of the Babies heat prickly powder ranged from 4.3×10^4 CFU/g to 1.32×10^5 CFU/g with sample 4 having the highest count of 1.32×10^5 CFU/g followed by sample 10 with a Total bacteria count of 1.03×10^5 CFU/g, then sample 3 had bacteria count of 1.01×10^5 CFU/g, with Sample 8 having a bacteria count of 6.1×10^4 CFU/g, while Sample 6 had the least count of 4.3×10^4 CFU/g. In the distribution of the bacteria isolated from Babies heat prickly powder samples. It was observed that *Staphylococcus aureus* was distributed across two (2) of the ten (10) samples analyzed then *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Bacillus sp* was found in only one sample each.

Table 3 The distribution and the total bacteria count of Babies heat prickly powder sample

Samples	Distribution and total bacteria count (CFU/g)			
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus sp</i>
Sample 1	-	-	-	-
Sample 2	-	-	-	-
Sample 3	-	-	-	1.01×10^5
Sample 4	1.32×10^5	-	-	-
Sample 5	-	-	-	-
Sample 6	-	4.3×10^4	-	-
Sample 7	-	-	-	-
Sample 8	6.1×10^4	-	-	-
Sample 9	-	-	-	-
Sample 10	-	-	1.03×10^5	-

Table 4 shows percentage occurrence of bacteria isolated from Babies heat prickly powder samples. It was observed that *Staphylococcus aureus* was the most frequently occurring isolate with 40% occurrence while *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Bacillus sp* had 20% in occurrence.

Table 4 Percentage occurrence of bacteria isolated from Babies heat prickly powder samples

Isolates	Number of occurrence	Percentage occurrence (%)
<i>Staphylococcus aureus</i>	2	40
<i>Staphylococcus epidermidis</i>	1	20
<i>Pseudomonas aeruginosa</i>	1	20
<i>Bacillus sp</i>	1	20
Total	5	100

Table 5 shows the antibiotics susceptibility pattern of the bacterial isolates from Babies heat prickly powder. Most isolates were sensitive to Ciprofloxacin (100%), Erythromycin (80%), Streptomycin (60%), Ampiclox (60%), Amoxicillin (60%), but resistant to Augmentin (40%), Gentamicin (20%), Septrin (20%), Chloramphenicol (20%) and Ofloxacin (20%).

Table 5 The antibiotics susceptibility pattern of the bacterial isolates from Babies heat prickly powder

Isolates	Number tested	Number of sensitive organisms (%)									
		SXT	CPX	APX	CN	AMX	S	E	CH	AU	OFX
<i>Staphylococcus aureus</i>	2	0(0)	2(100)	1(50)	0(0)	2(100)	1(50)	2(100)	0(0)	1(50)	0(0)
<i>Staphylococcus epidermidis</i>	1	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)
<i>Pseudomonas aeruginosa</i>	1	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	1(100)
<i>Bacillus sp</i>	1	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)
Total	5	1(20)	5(100)	3(60)	1(20)	3(60)	3(60)	4(80)	1(20)	2(40)	1(20)

Key: SXT = Septrin, CPX= Ciprofloxacin, APX = Ampiclox, CN = Gentamycin. AMX= Amoxicillin, E= Erythromycin, CH= Chloramphenicol, AU = Augmentin, OFX = Ofloxacin, S=Streptomycin

4. Discussion

Babies' heat prickly Powders are astringent powder. These powders also contains antibacterial, Absorbent and refreshing natural essential oil that aid in the relieving itching, heat rash and skin irritation on babies skin from hot weather. Babies Heat prickly powders were screened for their bacterial properties in this study. The results of this study revealed that 50% of the Babies heat prickly powders used in this study harbors bacteria species. The four isolates which occurred in most of the samples respectively sample 3, 4, 6, 8 and 10 were isolated, characterized, studied and eventually identified as belonging to the different genus *Bacillus*, *Staphylococcus*, *Pseudomonas*. Based on their respective characteristics as recommended in the Bergeys Manual of Determinative Bacteriology, they were further shown to belong to the species of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The results obtained show that the bacterial load of Babies heat prickly powder ranged from 4.3×10^4 to 1.32×10^5 CFU/g. The international Microbiological standards recommended limits for bacterial contamination in cosmetic products are 1.0×10^3 CFU/g for bacteria indicating that high bacterial load was observed in the present study. The frequency of occurrence of bacteria in the samples shows that not all the samples were contaminated with bacteria but indicating that most babies heat prickly powders can permit the growth of bacteria. It was also observed that gram positive organisms were the predominant contaminants in the powders which is in agreement with Hugbo et al., (2003) who reported that *Staphylococcus* spp. and other gram-positive cocci were the most predominant; gram negative isolates were hardly found. This leads to a presumption that the powders harbours more of Gram-positive bacteria than Gram negative bacteria. The results reported in this study is in agreement with Michael Macvren Dashen et al., (2011) who isolated *Staphylococcus aureus*, *Clostridium tetani* and *Bacillus* spp. while Ashour et al., (1989) reported that *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter agglomerans* and *Citrobacter freundii* were isolated. Omorodion et al., (2014) isolated *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Micrococcus* spp. and *Escherichia coli*. Choubey et al., (2017) isolated *Citrobacter* spp, *Staphylococcus aureus*, *Enterobacter* spp. The slight differences in the

results obtained may be due to poor storage, manufacturing practice or handling. Some of the organisms isolated have been implicated as causative agents of gastroenteritis. The percentage occurrence of the isolates from the baby powders were *Staphylococcus aureus* 40%, *Staphylococcus epidermidis* 20%, *Pseudomonas aeruginosa* 20%, and *Bacillus specie* 20%. Isolation of *Bacillus sp* that is free living is an indictment of raw materials used as well as the conditions prevalent on the environment in which the products were manufactured and packaged (Omorodion et al, 2014). While the isolation of *Staphylococcus spp.* is a function of personal hygiene on the part of the personnel producing the products since skin is the natural habitat of the organism. *Pseudomonas aeruginosa* is a function of improper hygiene. *Bacillus sp.* and *Staphylococcus spp.* in cosmetic products causes skin irritation. Generally, the results obtained from this study showed that most of these Babies heat prickly powders were highly contaminated. Isolation of bacteria as observed in this study could be caused by air contamination, poor hygiene, contaminated raw materials or poor manufacturing practice and improper storage.

5. Conclusion

This work was centered on the isolation and the Characterization of bacteria isolated from babies heat prickly powders. The results obtained in this work showed that five out of ten samples of the baby powders are capable of causing health hazards due to the presence of high bacteria load in the samples, this may be due to poor manufacturing practices, poor hygiene, contaminated raw materials or the susceptibility of the ingredients contained in the baby prickly heat powders. The presence of organisms such *Bacillus*, *Staphylococcus*, *Pseudomonas* in the baby powder samples implies that they can serve as vehicles for the transmission of disease. The need to control bacteriological contamination of the products has been of considerable concern to cosmetic manufacturer, so therefore, Good Manufacturing Practice (GMP) should be strengthened.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicting interests.

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