CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The use of headphone has been thought to cause infection in the ear canal and contribute to hearing loss. Commonly the headphone user also express concern regarding the potential for this device to cause noise- induced hearing loss. However, documented studies on the side effects of prolonged use of the headphone are rarely described in the literature (Mazlan et al., 2002). Increasing functionality and affordable prices for cell phones and smart phones have resulted in a global reliance on staying connected. Cell phones are now common place, whether in the dinner table, the kitchen, a restaurant, the gym, or even the bathroom. These factors and the heat generated by cell phones contribute to harboring bacteria on the device at alarming levels. When consider a cell phone's daily contact with the face, mouth, ears, and hands, the direct health risks of using germ-infested mobile devices are obvious. Wearing headphones or earplugs has been suggested as a possible predisposing factor for external ear canal infection since their use can increase the temperature and humidity of the canal, create the potential for skin abrasion and provide a vehicle for the introduction of organisms into the canal skin (Abbinay and Bharathi ., 2012). Isolation of bacterial agents from electronic devices such as hand-held computers and personal digital assistants has shown these devices to be possible modes of transmission of nosocomial pathogens (Bures et al., 2000).

During every phone call, the mobile phone ,and mobile phone headsets when used, come into close contact with strongly contaminated human body areas with hands to hands, and hands to other areas like mouth, nose and ears (Elkholy and Ewees ., 2010). As mobile phones act as perfect habitat for microbes to breed, especially in high temperature and humid conditions, they may serve as reservoirs of microorganisms that can easily transmitted from mobile phones to hands and therefore facilitate the transmission of bacterial isolates from one person to another (Srikanth *et al.*, 2008).

Microbial contamination is most commonly found on the mouthpiece and earpiece. While indirect contamination from person to person has decreased with the decline in the use of public payphones, cell phones with buttons and keyboards and other personal mobile phones in general has been found to be even more conductive to bacterial contamination (Lee *et al.*, 2013).

Rusin, and his colleagues had documented both Gram-positive and Gramnegative bacteria in hand-to-mouth transfer during casual activities. This implies that mobile phones may serve as vehicles of transmission of diseases such as diarrhoea, pneumonia, boils, and abscesses (Rusin *et al.*, 2002).

Unlike our hands, which are easily sterilized, our mobile phones are cumbersome to clean. Even we rarely make an effort to sanitize them. As a result, these devices carry a variety of bacteria. Cellphones are used often in hospitals by patients, visitors, and health care workers. Also, travelers who go to low-income countries where potable water and good sanitation are limited are exposed to the risk of contracting infections because these individuals carry phones, and the potential of such accessories to spread bacterial infection is not yet clear (Brady *et al.*, 2006).

1.2. Rationale

Increasing functionality and affordable prices for mobile phones and smart phones have resulted in a global reliance on staying connected.

Due to a few literature and in availability of data about this topic in Sudan, this dissertation is conducted for the purpose to provide a data about bacterial contamination of mobile phone headsets.

1.3. Objectives

1.3.1. General Objective

1- To assess Gram- positive bacterial contamination on mobile phone headsets among universities students.

1.3.2. Specific Objectives

1- To collect samples from mobile phone headsets.

- 2- To determine the bacterial load on mobile phone headsets.
- 3- To identify Gram- positive that are bacteria found on mobile phone headsets.

CHAPTER TWO

LITERATURE REVIEW

The global system for mobile telecommunication was established in 1982 in Europe with a view of providing an improved communications network. The first use of mobile phones in India was in 1995; today there are 287 million mobile phone users in India, which accounts for 85 percent of all telecommunication users (Kapdi *et al.*, 2008). Today, mobile phones have become one of the most indispensable accessories of professional and social life. Although they are usually stored in bags or pockets, mobile phones are handled frequently and held close to the face (Neubauer *et al.*, 2005).

The first study of bacterial contamination of mobile phones was conducted in a teaching hospital in Turkey with a bed capacity of 200 and one intensive care unit (Karabay *et al.*, 2007). One-fifth of the cellular telephones examined in a study conducted in New York were found to harbor pathogenic microorganisms. Health care workers' mobile phones provide a reservoir of bacteria known to cause nosocomial infections. UK National Health Service restrictions on the utilization of mobile phones within hospitals have been relaxed; however, utilization of these devices by inpatients and the risk of cross-contamination are currently unknown (Goldblatt *et al.*, 2007).

Wearing headphones or earplugs has been suggested as a possible predisposing factor for external ear canal infection since their use can increase the temperature and humidity of the canal, create the potential for skin abrasion and provide a vehicle for the introduction of organisms into the canal skin. In a previous study that was conducted to determine the association of constant and frequent use of earphones with bacterial growth inside the ear and the potential role of earphones as vector of commensals, fifty male medical students, who were not yet exposed to the hospital environment, volunteered for the project as healthy individuals. Each volunteer signed an informed consent form and filled up a questionnaire. Observable signs of infection that were looked for in the ear canal include swelling and reddening of the ear canal, discharge and foul odour of the ear canal. Symptoms included itching, pain and tenderness upon manipulation of the pinna and feeling of fullness in the ear. Only such students were included in the study who did not have any such signs or symptoms (Mukhopadhyay *et al.*, 2008).

Swabs were collected from the left ear and the left ear piece of the earphone. They were divided into two groups of 25 each. Group A subjects were students of ages 18 to 25 years who infrequently use or never use earphones. In this group, the swabs (ear and earphones) were collected at random. The group B subjects were students aged 18 to 25 years who used to listen music with earphone constantly and always at a stretch of at least 30 minutes. The swabs (ear and earphones) were collected immediately after earphone usage. The swabs were inoculated in sheep blood agar and incubated aerobically overnight at 37°C and the bacteria were identified according to the recommended procedure. The different variables were tested and the test of significance was done using chi square test. A total of 43 ear samples (86%) and 31 earphone samples (62%) showed bacterial growth, ranging from very scanty to heavy. In Group A, 20 samples (80%) from ear and 14 samples (56%) from earphones, and in Group B, 23 samples (92%) from ear and 17 samples (68%) from earphones had growth (Mukhopadhyay *et al.*, 2008).

In a study conducted in Queen Elizabeth Hospital in Barbados, West Indies, more than 40 percent of mobile phones of 266 medical staff and students were culture positive (Ramash *et al*., 2008). Ulger, *et al*, reported that 94.5 percent of 200 health care workers and their mobile phones were contaminated with various microorganisms, including nosocomial pathogens, in a study conducted in New York and Israel (Ulger *et al.*, 2009).

Researchers conducted a pilot study to estimate the prevalence and type of microorganisms isolated from the mobile phones of 80 health care workers at a Thai hospital before and after alcohol cleansing. The surface of the phone's keypad, mouthpiece, and earpiece was swabbed, and the phone was cleaned with a 70% alcohol pad. A second culture swab of the keypad, mouthpiece, and earpiece was obtained one minute later. The researchers reported that 38 participants (47.5%) had exposure to multidrug-resistant bacteria at enrollment in the study, and there was an average of two cases per house staff with multidrug-resistant bacteria. Three mobile phones (3.8%) had cultures positive for *Acinetobacter* spp. before alcohol cleaning. After alcohol cleansing, no microorganisms were detected. Overall hand hygiene compliance was 39% before touching a patient, 29.4% before a clean/aseptic procedure, and 47.5%

health care workers' mobile phones as a reservoir for various multidrugresistant bacteria, none had shown that alcohol cleansing can reduce the detection of bacteria on mobile phones (Sumritivanicha *et al.*, 2011).

A cross-sectional study was conducted in Turkey to determine bacterial colonization on the mobile phones used by patients, patients' companions, visitors, and health care workers. Significantly higher rates of pathogens (39.6% versus 20.6%, respectively; P = 0.02) were found in mobile phones of patients' (n = 48) versus the health care workers (n = 12). There also were more multidrug pathogens in the patients' mobile phones, including methicillinresistant Staphylococcus aureus, extended-spectrum ß-lactamase-producing Escherichia coli, Klebsiella spp, high-level aminoglycoside-resistant Enterococcus spp, and carabepenem-resistant Acinetobacter baumanii. Findings suggest that mobile phones of patients, patients' companions, and visitors represent higher risk for nosocomial pathogen colonization than those of health care workers. Specific infection control measures may be required for this threat (Tekerekoglu et al., 2011).

A cross-sectional study was conducted in India to determine the level and type of bacterial contamination of the mobile phones of dental personnel involved in direct patient care and to determine the usefulness of cleaning with 70 percent isopropyl alcohol for decontamination. Dental faculty and trainees in an Indian dental school were asked to participate in a study in which a questionnaire was administered concerning patterns of mobile phone use and disinfection. Swabs from mobile phones of the participants were taken using moist sterile swabs and plated on blood agar plates. The bacteria isolated were identified by biochemical tests. Eighteen percent of the participants (n=9) reported using their phones while attending patients. Nearly 64 percent (n=32) used their mobiles for checking time, and 64 percent (n=42) reported never cleaning their phones. In total, 50 mobile phones were cultured for microorganisms: 98 percent (n=49) were culture-positive, and 34 percent (n=17) grew potentially pathogenic bacteria. There was significant reduction in the mean number of colony-forming units after decontamination with alcohol (p less than 0.001). The bacterial load was reduced by around 87 percent. The results of this study showed that mobile phones may act as an important source of nosocomial pathogens in the dental setting. Therefore, it is important for dental school administrators to encourage higher compliance with hand-washing practices and routine surface disinfection through framing of strict protocols to reduce the chances of occurrence of nosocomial infections (Singh *et al.*, 2010).

In Nigeria, there has been an increase in the use of mobile phones among the general population, and the use of phones is common in certain areas of the environment where the percentage presence of bacteria is likely high, such as in hospitals, in animal slaughter areas, and in toilets. A study was conducted to determine whether mobile phones could play a role in the spread of bacterial pathogens and to offer possible control or preventive measures that could be instituted to avoid this likely vehicle of infection. In this study, 62 percent of 400 mobile phones from all of the study groups were found to be contaminated

by bacterial agents. Isolation of bacterial agents from electronic devices such as hand-held computers and personal digital assistants has shown these devices to be possible modes of transmission of nosocomial pathogens (Bures *et al.*, 2000).

A study was conducted in Turkey to determine the contamination rate of health care workers' mobile phones and hands in operating rooms and ICUs. These results showed that HCWs' hands and their mobile phones were contaminated with various types of microorganisms (Ulger *et al.*, 2009).

A study was conducted in Southern India to determine whether mobile phones of health care workers (HCWs) and corporate users harbor micro-organisms. Swabs collected from mobile phones were inoculated in solid and liquid media aerobically. Growth was identified as per standard and incubated microbiological procedures. Antibiotic susceptibility was determined for Staphylococcus aureus. A questionnaire was used for data collection on awareness of mobile phone use. Of 51 HCWs and 36 corporate mobile phones sampled, only five (6%) showed no growth. Pathogens isolated from HCW samples included coagulase-negative Staphylococci, S. aureus (methicillinsensitive S. aureus, methicillin-resistant S. aureus), Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Among corporate isolates, 29% were pathogenic. Polymicrobial growth was detected in 71% of HCW mobile phones and 78% of corporate mobile phones. Only 12% of HCWs used disinfectants to wipe their mobile phones. Therefore, it was concluded that mobile phones serve as a ready surface for colonization of nosocomial agents, indicating the importance of hand hygiene to prevent cross-transmission (Srikanth *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross- sectional study.

3.1.2. Study area

The study was conducted among university students in Khartoum State. The students studied in Sudan University for Sciences and Technology (SUST), University of Khartoum (U of K), The National Ribat University, Bahry University, Al Neelain University, Omdurman Islamic University, Africa International University, and Albian College who use mobile phone headsets were randomly selected. The practical part of this study was carried out in the Research Laboratory, SUST.

3.1.3 Duration of the study

The practical part of this study was done in the period from April to May 2014.

3.2 Ethical consideration

All the students enrolled were agreed to participate in the study before collecting samples from their mobile phone headsets.

3.3. Samples collection and dispatch

The ear phone was sampled by a sterile cotton wool swab. The swab was moistened with a sterile normal saline just before rubbing the swab on the surface of one of the ear phone headset. The swabs were put in a sterile containers each containing 2 ml of sterile normal saline. The collected swabs were transported to the Research Laboratory within half an hour.

3.4. Enumeration of bacterial load

The enumeration of bacterial load was carried out using pour plate method. The method was performed as follows :

A clean, sterile, dry pipette was used to transfer 1 ml of sample to tube 1 which containing normal saline and was mixed thoroughly. Notice that tube 1 now contains 1/10 the concentration of bacteria in the original sample .

Using another clean, sterile, dry pipette 1 ml was removed from tube 1 and transferred to tube 2 and mixed thoroughly.

The serial dilution method was continued till reaching tube 6.

1 ml from tubes 4,5, and 6 were plated by the pouring plate method by pouring 15 ml of sterile nutrient agar and mix it with the 1 ml dilution gently. Three plates were used for each dilution. The plates were incubated at 37 for 48 hours.

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3.4.2 Calculations

At the end of incubation period, bacterial load was done in the term of colony

forming unit per ml (CFU/ml) according to the following formula:

CFU * DF * 1/ aliquot = CFU/ ml

CFU: Colony Forming Unit.

DF: Dilution Factor.

3.5. Identification of Gram-positive bacteria

3.5.1. Colonial morphology

The samples were inoculated on blood agar and MacConkey agar and both plates were Incubated aerobically at $35 - 37^{\circ}$ C overnight. After incubation, the colonial morphology of the isolates were reported.

3.5.2 Gram's stain

An evenly spread smear of the isolate was made on a clean, dry slide using a sterile normal saline and the smears were allowed to air- dry in a safe place.

The smears were fixed by passing three times over Bunsen flame and stained by the Gram technique as follows;

The smear was covered with crystal violet stain for 30 - 60 seconds. After that, the stain was rapidly washed off with clean water. All the water was tipped out, and then Lugol's iodine was added for 30 - 60 seconds. The iodine was washed off with clean water. Then, the smear was decolorized rapidly (few seconds) with acetone – alcohol and washed immediately with clean water. After that, the smear was covered with neutral red stain for 2 minutes. The stain was washed off with clean water. The back of the slide was wiped clean, and the slide was placed in a draining rack for the smear to air- dry. The smear was examined microscopically microscopically, first with the 40X objective to check the staining and to see the distribution of material, and then with the oil immersion objective to look for bacteria and cells. The condenser iris was opened fully when using the oil immersion lens.

3.5.3 Biochemical tests

The biochemical reactions was performed to identify the isolated bacteria. While several commercial systems for identifying bacteria are available, these are often difficult to obtain or too expensive to use in developing countries. These commercial biochemical tests was adopted as follows, (Cheesbrough M., 2000).

3.5.3.1 Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non- catalase producing bacteria such as streptococci.

About 2-3 ml of 3% hydrogen peroxide was poured into a test tube, by mean of a sterile wooden stick, a good growth of the test organism was removed and immersed in the hydrogen peroxide solution. Immediate bubbling in the solution indicate positive test.

3.5.3.2 Coagulase test

This test is used to differentiate *Staphylococcus aureus* (*S. aureus*) which produce the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase.

A drop of physiological saline was placed on a slide. A colony of the test organism was emulsified in the drop to make a thick suspension. A drop of plasma was added to the suspension and gently mixed. Clumping of the organisms within 10 seconds indicate positive test.

3.5.3.3 DNase test

This test is used to differentiate *S. aureus* which produce the enzyme DNase from other staphylococci which do not produce DNase. It is particularly useful if plasma is not available to perform a coagulase test or when the results of a coagulase test are difficult to interpret.

Using a sterile loop, the test organisms were spot-inoculated on DNase agar medium. The plate was incubated at 35-37°C overnight. The surface of the plate was covered with 1 mol/l hydrochloric acid solution. The excess of acid was tipped off. Clearing around the colonies was looked within 5 minutes of adding the acid to indicate positive test.

3.5.3.4 Fermentation of Sugars

This test was performed in a sugar broth medium to test an organism's ability to ferment sugars as well as its ability to produce gas and H_2S . The medium is contains PH indicator Andrade which converts to the red color in the acidic PH indicating sugar fermentation. The sugars were glucose, mannitol, lactose and sucrose.

A suspension of the tested isolate was prepared using a sterile normal saline. Then each sugar broth (contains an inverted Durham tube) was inoculated separately with the prepared suspension using a sterile wire loop. The tubes were incubated at 35-37°C for 24 hours. A change in the color to red indicating fermentation of the certain sugar found in that tube (Johnson and Case., 2007).

3.6 Data analysis

The collected data were analyzed to calculate the (P - value) using SPSS computer program version 11.5.

CHAPTER FOUR

RESULTS

A total number of 200 mobile phone headsets were sampled to determine bacterial load and to identify Gram- positive bacteria isolated from these headsets. The samples were taken from the mobile phone headsets of the students belonging to SUST 30 (15%), U of K 30 (15%), Al Neelain University 30 (15%), Omdurman Islamic University 30 (15%), Bahry University 20(10%), The National Rebat University 25 (12.5%), Albian College 15 (7.5%), and Africa International University 20 (10%) (Table1). 140 samples (70%) were taken from the mobile phone headsets of female students and 60 samples (30%) were taken from the mobile phone headsets of male students, (Table 2). The range of the bacterial load in this study was calculated and found to be $8-30\times10^7$ /ml. Eleven samples (5.5%) showed bacterial growth and nine of them were Gram- positive bacteria (Table 3). These were 6 (66.7%) *Staphylococcus aureus*, 2 (22.2%) *Staphylococcus epidermidis*, and the last isolate (11.1%) and 1 *Bacillus* sp. (Table 4).

The distribution of the isolates according to universities was as follows, 3 (33.3%) from U of K, 2 (22.2%) from headsets of students in SUST, 1 (11.1%) from Omdurman University, 2 (22.2%) from Al neelain University and 1 (11.1%) from the mobile phone headsets of students in Bahry University, (Table 4). From these 9 Gram- positive isolates, 6 (66.7%) were

isolated from the mobile phone headsets of the female students and 3 (33.3%) were isolated from the mobile phone headsets of the male students. The study showed that there is no difference in the contamination of mobile phone headsets between male and female students (p= 0.89).

The results of the biochemical tests performed to identify the isolates were as follows:

All *Staphylococcus. aureus* isolates were produce catalase, coagulase, DNAse enzymes and ferment glucose, mannitol, sucrose and lactose sugars with gas production. All *Staphylococcus. epidermidis* isolates produce catalase enzyme but neither coagulase nor DNAse enzymes and they didn't ferment mannitol sugar. The Bacillus sp were identified microscopically, Gram- positive sporeforming rods, and it is haemolytic on Blood Agar under aerobic conition.

University	Number of specimens
U of K	30
SUST	30
Al neelain University	30
Bahry University	20
Omdurman Islamic University	30
The National Rebat University	25
Albian College	15
Africa International University	20
Total	200

Table 1. Distribution of samples according to universities

T T	Ge	Total	
University	Males	Females	
U of K	13	17	30
SUST	7	23	30
Al neelain University	10	20	30
Bahry University	5	15	20
Omdurman Islamic University	14	16	30
The National Rebat University	6	19	25
Albian College	3	12	15
Africa International University	2	18	20
Total	60	140	200

Table 2. Distribution of samples according to gender

Table 3. Gram- positive isolated from different mobile phone headsets ofuniversities students

University	N. of specimens	N. of Gram- positive isolates
U of K	30	3
SUST	30	2
Al neelain University	30	2
Bahry University	20	1
Omdurman Islamic University	30	1
The National Rebat University	25	0
Albian College	15	0
Africa International University	20	0
Total	200	

Table 4:	Gram-	positive	species	isolated
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University	No of Gram- positive isolates	Name of Gram- positive isolates
U of K	3	S. aureus (n=3)
SUST	2	S. aureus (n=2)
Al neelain University	2	Bacillus sp (n=1) S. epidermidis (n=1)
Bahry University	1	S. aureus (n=1)
Omdurman Islamic University	1	S. epidermidis(n=1)
The National Rebat University	0	-
Albian College	0	-
Africa International University	0	-

CHAPTER FIVE

DISCUSSION

5.1. Discussion

The using of mobile phone headsets becomes widely common among people around the world. Most of them are unaware or always forget cleaning their devices which can harbor different types of microbes. Isolation of bacterial agents from electronic devices such as mobile phones and personal digital assistants has shown these devices to be possible modes of transmission of nosocomial pathogens (Bures *et al.*, 2000). In the present study, only 5.5% of the total samples were positive for bacterial growth. In a study conducted in Queen Elizabeth hospital in Barbados, West Indies, over 40% of mobile phones of 266 medical staff and students were culture positive for bacterial growth (Ramesh et al, 2008) which is higher than our study. Ulger et al. (2009) reported that 94.5% of 200 health care workers and their mobile phones were contaminated with various microorganisms. Another study which was conducted to determine the association of using earphones with bacterial growth showed that 68% of samples from earphones had growth (Mukhopadhyay et al., 2008). In comparison with the studies mentioned above, our study showed a very low percentage of bacterial growth (5.5%). The present study was done in the period from April to May 2014 which are the months of the summer season in Sudan characterized by very hot and dry weather that may be responsible for the low result obtained in this study. Most

of studies were done on the mobile phones of patients, health workers and hospital visitors. All of these study groups became in contact with microbes especially the nosocomial microorganisms explaining the high results obtained in the previous studies. When the types of organisms isolated are considered, despite the difference in the isolation rate, most studies had reported similar types of organisms. *S. aureus* and coagulase- negative staphylococci were the most frequently isolated organisms in those studies which is in line with the present study.

5.2. Conclusion

1. Contamination of mobile phone headsets examined was very low.

2. Potentially pathogenic Gram- positive bacteria were isolated from mobile phone headsets.

5.3. Recommendations

1. Education campaigns of hygienic practices is highly recommended for the university students to increase their awareness about bacterial contamination of mobile phones and devices.

2. Routine cleaning of mobile phones and their devices may be effective in reducing bacterial contamination.

3. In the future, mobile phones could be produced with protective material against bacterial contamination.

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Appendix (1) culture media

1.1. Preparation of culture media

- 1. Agar medium should be prepared from a commercially available dehydrated base according to the manufacture instructions.
- 2. Immediately after autoclaving, allow it to cool in $a45-50C^0$ water bath.
- 3. Pour the freshly prepared and cooled medium into glass or plastic, flatbottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4mm. this correspond to 60-70ml of medium for plate with diameter of 150mm.
- 4. The agar medium should be allowed to cool to room temperature and unless the plate is used the same day, store in refrigerator $(2 \text{ to}8\text{C}^0)$.
- 5. A representative sample of each batch of plates should be examined for sterility by incubating at $30-35C^0$ for 24 hours or longer.
- 6. Inoculate the sample directly and incubate it at $37C^{0}$ for 24 hours.

1.2. DifcoTMNutrient Agar

Approximate formula * per Liter

Beef Extract	3.0g
Peptone	5.0g
Agar	15.0g

*Adjusted and \or supplemented as required to meet performance criteria.

1.3. DifcoTMMacConkey Agar

Approximate formula * per Liter

Peptone	20.0g
Lactose	10.0g
Bile Salts	5.0g
Sodium Chloride	5.0g
Agar	12.0g
Nutrient Red	0.05g

1.4. DifcoTMManitol Salt Agar

Approximate formula * per Liter

Proteose Peptone No.3	10.0g
Beef Extract	1.0g
D-Mannitol	10.0g
Sodium Chloride	75.0g
Agar	15.0g
Phenol Red	25.0g

Appendix (2) Instruments

2.1 Safety cabinet Daihan lab tech co LTD, made in UK.

2.2 Incubator GALLENKAMP Made in UK

2.3 Freezer-20 Made in EUROP

2.4 Water bath Model: LWB-111D, made in UK

2.5 Microscope Model A15120-4, made in Germany

2.6 Sensitive balances

2.7 Ultra low temperature freezer-70

Model MDF-392, made in Japan

2.8. Refrigerator with glass door

Made in Saudi Arabia

2.9 Autoclave

Dixons, surgical instrument LTP, made in UK