CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of the study

This is a cross-sectional study conducted to assess Gram-negative bacteria on computer keyboards in universities at Khartoum State.

3.1. 2. Study area

The study was conducted in Sudanese universities at Khartoum State includes Sudan University of Science and Technology, Al-Neelain University, University of Science and Technology and Omdurman Ahlia University.

3.1.3. Study duration

The study was conducted in the period from March to August 2014.

3.2. Collection of samples

Samples were collected randomly from keyboards of multiple user computers. Sterile cotton swabs moisten in sterile normal saline (Appendix 8) were wiped firmly over the entire surface of the keyboards. Each swab was placed in 2ml sterile normal saline.

3.3. Bacterial load

The bacterial load was calculated using pour plate method. A measured amount of the suspension of living bacteria is mixed with molten agar (Appendix 2) medium in a Petri dish. After incubation, the number of colonies was counted. Count of pure cultures should be made on plates incubated at 37°C over-night. Plates yielded colonies between 30-300 were counted to determine bacterial load.

A serial 10-fold dilution of the bacterial suspension was prepared, 9ml of normal saline was pipetted into each several sterile test tubes, 1ml of suspension was transferred into the first tube of diluents. The first dilution was mixed with sterile pipette and then 1ml was transferred to the next diluents, 1ml of each the greatest dilutions were pipetted into each three Petri dishes, 15ml of clear nutrient agar was poured to 1ml of suspension in Petri dish. Mixed, allow to cooled and incubated at 37°C over night, the preferred range for total CFU/plate is between 30-300 colonies/plate were counted (Collee *et al*., 1996).

3.4. Identification of the isolates

The suspected organisms were sub cultured on macCkonkey agar (Appendix1) and blood agar (Appendix 3) for identification.

3.4.1. Gram's stain

the fixed smear was covered with crystal violet stain (Appendix 9) for 30-60 second, the stain was cleaned with water, the smear was covered with lugol's iodine(Appendix 9) for 30-60 seconds, the iodine stain was washed with clean water, the stain was decolorized with absolute – alcohol (Appendix 9) for few second, washed immediately with clean water, the smear was covered with safranin (Appendix 9) for 2 minutes, the stain was washed with clean water, the back of the slide was cleaned and placed in draining rack for the smear to air dry, the smear was examined microscopically, with oil immersion objective to report the bacteria and cells (Cheesbrough, 2006).

3.4.2. Biochemical tests

3.4.2.1. Citrate utilization test

The test based on the ability of an organism to use citrate as its only source of carbon. Koser's citrate medium was used (Appendix 5), the organism under test was inoculated and incubated over- night at 35- 37°C. Result were positive (Bright blue), negative (no change in color of media) (Cheesbrough, 2006).

3.4.2.2. Urease test

The under test organism was inoculated in bottle containing 3ml sterile Christenen's modified urea agar (Appendix 7) (Oxoid Company) then incubated at 35-37°C for 3-12 hr. positive result give pink color, no pink color negative result (Cheesbrough, 2006).

3.4.2.3. Indol test

The under test organism was inoculated in 3ml sterile peptone water (Appendix 6), incubated at 35-37°C for up to 48h then to test indol 0.5 ml kovac's reagent (Appendix10) was added, shaked gently, examined for ared color in surface layer with in 10 min (Cheesbrough, 2006).

3.4.2.4. Sugar fermentation and production of H_2S and gas

The under tested organism was inoculated into kligler iron agar(Appendix 4), Incubated at 35-37°C and then examined after 24 hours for lactose and glucose

fermentation, acid production was indicated by appearance of yellowish color, while gas production was indicated by presence of empty space in the test tube, and the production of H_2S was indicated by blackening of the media (Cheesbrough, 2006).

3.5. Preservation of cultures

Cultured organisms were preserved in nutrient agar slope at 4°C.