Klebsiella pneumoniae: A potential food safety risk in wild fruits and dried vegetables in Botswana

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Abstract

The bacterium *Klebsiella pneumoniae* is ubiquitous in nature and is found on the general vegetation and their fruits. The subspecies "*pneumonia"* is known to cause community-acquired and nosocomial pneumonia in immune-compromised persons (Ko *et al*., 2002; Yu *et al*., 2007; Yinnon *et al*., 1996 and Struve and Krogfelt, 2004). Some the vegetation and wild fruits form part of the staple diet or is consumed as delicacies in Botswana. The potential presence of *K. pneumoniae* therefore poses a potential food safety hazard given the fact that the gastro-intestinal route is considered one of the major infection routes of *K. pneumoniae* (Struve and Krogfelt, 2004 and Struve and Krogfelt, 2003). This study sought to evaluate the occurrence of *K.*

pneumoniae in dried vegetables and fruits consumed in Botswana. One representative dried vegetable (*morogo wa dinawa*) and two representative wild fruits (*morula* and *moretlwa*) were selected for the study based on their popularity in the country. The study confirmed the occurrence of *K. pneuminioniae* on the three foods studied. The isolation rates of the microbe per sample type was 92.3% (*morula* fruit), 86.4% (*morogo wa dinawa*) and 86.2 (*moretlwa*). The overall isolation rate from the three sample types was 89.3%. This showed that there is a significant presence of the microbe on the three foods studied therefore there is a potential food safety risk in consuming these foods by susceptible groups (immune-compromised individuals). The API20E identification system showed five different biotype profiles. The most frequent API20E biotype encountered was 5215773 (44.6%) followed by 5214773 (39.1%). The rest of the biotypes (5215573, 5015773 and 4215773) occurred at a frequency of 2.2%. These biotypes show that there are definite biochemical differences between the isolates. Of the isolates obtained 38% were of the encapsulated phenotype. The highest in-sample occurrence rate of the encapsulated phenotype was in *morogo wa dinawa* (52.6%) followed by *moretlwa* (40%) and lastly *morula* (31.3%). Antibiotic susceptibility testing by the disk diffusion method showed that the isolates were most susceptible to 30ug cefotaxime (24.4mm average clearance zone), and totally resistant to 1ug oxacillin and 30ug vancomycin (0.0mm average clearance zone). All of the strains analysed were completely resistant to oxacillin (1ug) and vancomycin (30ug). For cotrimoxazole (25ug), 22.8% of the isolates were completely resistant.

Key Words: *Klebsiella pneumoniae*, nosocomial infection, community-acquired infection, bacteremia, septicaemia.

1. Introduction

The bacterium *Klebsiella pneumonia* is ubiquitous in nature and is found in the general environment, surface waters, soil, plants and other vegetation (Podschun *et al*., 2001). The bacterium belongs to the genus *Klebsiella* and the family *Enterobacteriaceae.* It is a well-known causative agent for nosocomial infections (also called hospital-acquired infections) and, recently, community-acquired bacterial pneumonia (Haryani *et al*., 2007). The species *Klebsiella pneumoniae* is medically the most important species of the genus. It is specifically associated with pneumonia, bacteremia, septicaemia, urinary tract infections, chronic pulmonary disease, soft tissue infections and diarrhea (Haryani *et al.*, 2007). The nosocomial infections caused by *K.*

pneumoniae are characterized by multi drug resistance and high mortality (Struve and Krogfelt, 2004; Matsen *et al*., 1974). The community-acquired bacterial pneumonia caused by the bacterium affects primarily people with impaired host defenses, for example patients with diabetes mellitus, chronic alcoholics, malignancy, renal failure and chronic obstructive pulmonary disease (www.uptodate.com/contents/overview-of-klebsiella-pneumoniae-infection?vie).

The ubiquitous nature of *K. pneumoniae* and the seriousness of the consequences of infection by the microbe for susceptible subjects gives it the status of an important emerging pathogen. The possible presence of *K. pneumoniae* in or on foods could therefore pose a serious public health risk specifically in the susceptible groups. Foods that may harbor the bacterium include indigenous leafy vegetables and wild fruits. In Botswana, many naturally occurring vegetables and fruits are consumed as part of the traditional dishes or for leisure. Vegetables consumed include “morogo wa dinawa”, “lothue” and “thepe” to name a few. Wild fruits consumed locally include “moretlwa” and “morula”. The gastro-intestinal tract is recognized as an important route of *K. pneumoniae* infections (Struve and Krogfelt, 2004 and Struve and Krogfelt, 2003) therefore it is very important to investigate the occurrence of the bacterium in the foods mentioned and to evaluate their relationship to isolates obtained from humans in the clinical setting.

1.1 Statement of the problem

The bacterium *Klebsiella pneumoniae* is ubiquitous in nature including vegetation and wild fruits some of which are consumed as food in Botswana (Struve and Krogfelt, 2004).The subspecies “*pneumoniae”* is known to cause community-acquired and nosocomial pneumonia in immune-compromised persons (Ko *et al*., 2002; Yu *et al*., 2007; Yinnon *et al*., 1996 and Struve and Krogfelt, 2004).This implies that isolates from food sources may, under appropriate conditions, directly cause community-acquired pneumonia or serve as a reservoir that feeds nosocomial infections in hospitals through interaction between hospital patients and the general public. It is therefore important to establish the occurrence rates of the bacterium in the suspect foods and to characterize such isolates in terms of their potential to cause infection as well as their antibiotic resistance patterns.

1.2 Significance of Study

The study seeks to evaluate the occurrence rates of *K. pneumoniae* in a dried vegetable (*morogo wa dinawa*) and two wild fruits (*morula* and *moretlwa*) widely consumed in Botswana by both urban and rural folks. This information is important in appraising the extent of public health risk posed by this microbe. It also helps to inform the formulation of processing parameters and behaviors of those involved in the processing of the suspect foods. Healthcare practices could also benefit by getting a better understanding of the relationship between food and hospital isolates/strains of *K. pneuminioniae*.

1.3 Justification of Study

Epidemiological studies have shown that *K. pneumoniae* infections are frequently preceded by gastrointestinal colonization (Struve and Krogfelt, 2004). The bacterium is likely to be present in the foods of interest to the study. These foods are widely consumed in Botswana including by people most at risk of contracting *K. pneumoniae* infections (diabetics and alcoholics). Both these groups have a significant presence in Botswana (www.diabetesatlas.org/content/afr-data). It is therefore justified to carry-out this study to inform the formulation of food processing parameters and behaviors of those who come in contact with hospitalized patients.

1.4 Objectives of the study

1. To isolate and positively identify *K. pneumoniae* from "*morogo wa dinawa*" (dried vegetable), "*morula*" (wild fruit) and "*moretlwa*" (wild fruit).
2. To determine isolation rates of the microbe from the three food sources.
3. To determine the antibiotic susceptibility patterns of the isolates.
4. To compare the antibiotic susceptibility patterns of the food isolates to the clinical *K. pneumoniae* isolates.
5. To propose ways of reducing any potential food safety risks associated with the studied foods.

2. Literature Review

2.1 Incidence of *Klebsiella Pneumoniae*

As early as 1974, assumptions were made that *Klebsiella* were widely distributed in nature (Matsen *et al*., 1974). More recent studies have confirmed these earlier assumptions. Podschun *et al* (2001) reported that *Klebsiella* are found in the general environment, surface waters, soil, plants and vegetation. The genus is also found on mammalian mucosal surfaces of humans, horses and swine (Struve and Krogfelt, 2004; Podschun and Ullman, 1998). The genus is not common on the human skin because gram-negative bacteria generally do not grow well under the conditions provided by the human skin (Podschun and Ullman, 1998). The normal carrier rates of *Klebsiella* species in healthy individuals are reported by Podschun and Ullman (1998) to be 5-38% in stool and 1-6% in the nasopharynx. They report the carrier rates in hospitalized patients to be 77% in the stool, 19% in the pharynx, and 42% on the hands. The higher carrier rates in hospitalized patients are attributed to the use of antibiotics particularly the broad-spectrum or multiple antibiotics.

2.2 Taxonomy, Differentiation and Typing of *Klebsiella* Species

The adoption of a consistent nomenclature on Klebsiella has been complicated by the fact that there is no consensus in the developed world as to which of the available systems to adopt. Great Britain and the former Commonwealth countries adhere to the classification of Cowan whereas the USA and most European countries prefer that of Orskov (Podschun and Ullman, 1998). Consequently, the same bacterium may be called *K. pneumoniae* in one country and *K. aerogenes* in another, thereby creating confusion and lack of standardization which limits scientific communication between researchers in different parts of the world.

Klebsiella species are usually identified and differentiated according to their biochemical reactins (Podschun and Ullman, 1998). The genus is described as containing gram-negative, non-motile, usually encapsulated rod-shaped bacteria of the family *Enterobacteriaceae*. Whereas most of the Klebsiella species can be identified by standard microbiological laboratory tests, the species *K. terrigena* and *K. planticola* require special, non-conventional reactions such as utilization of hydroxyl-l-proline or growth at 10^{o}C.

It is often necessary to determine the clonality of strains involved in epidemic or endemic nosocomial outbreaks and various methods have been developed to do this. These include biotyping, serotyping, phage typing, bacteriocin typing and molecular typing (Podschun and Ullman, 1998).

2.2.1 Biotyping

Biotyping may be automated or manual. Manually, it can be carried out using macro-tubes alone or combining a commercial system with additional macro-tubes. All commercial identification systems are based on one of five different technologies or a combination thereof (O'Hara, 2005). These technologies are pH-based reactions, enzyme-based reactions, utilization of carbon sources, visual detection of bacterial growth and detection of volatile or non-volatile fatty acids by gas chromatography (O'Hara, 2005). In the pH-based reactions, a positive test is indicated by a change in color of one or more dyes. When a carbohydrate is used the pH becomes acidic and when a protein is used, the pH becomes alkaline due to the release of a nitrogen-containing compound. The reactions are influenced by inoculum size, incubation time and temperature of the reaction.

In enzyme-based reactions, color changes are due to the hydrolysis of a colorless complex by an appropriate enzyme with the resulting release of a chromogen (O'Hara, 2005). The incubation times needed for enzymatic assays are generally shorter than those required for pH-based reactions.

In the utilization of carbon sources there is a transfer of electrons from an organic product to the dye tetrazolium violet, which is incorporated within each test. This causes a colorimetric change in the dye showing the increased cellular respiration that occurs during the oxidation process. The reaction can occur in as little as 4hrs (O'Hara, 2005).

Visual detection of bacterial growth involves detection of increased turbidity in the presence of a substrate. The results are determined by comparing a control well with the test well (O'Hara, 2005).

Determination of volatile or non-volatile fatty acids is done using gas chromatography. The method is complex and therefore not commonly used. The method involves the detection of the

end products of cellular fatty acid metabolism (O'Hara, 2005). The end products are displayed on chromatographic tracings that are compared to a library of known patterns (O'Hara, 2005).

There are several manual identification systems including the API 20E, API RapiD 20E, Crystal E/NF, EPS, GN2 MicroPlate, ID Tri-Panel, ID 32E, Microbact, RapID on E, UID/UID-3, Enterotube, and Micro-ID. Of these, the API 20E has become somewhat of a "gold standard" among commercial systems and is largely accepted by the clinical microbiology laboratory market (www.biomerieux-usa.com/support/technlibrary/api/index.asp). The system is used for identifying *Enterobacteriaceae* and other Gram-negative rods. It consists of an impermeable plastic backing supporting 20 cupules that contain pH-based substrates. The cupules of the strip are inoculated with a suspension of the culture according to the instruction manual and incubated at 37^{o}C for 24-48hrs. Results are read and recorded onto a pre-designed record sheet which combines cupule reactions into sets of three with values 1, 2 and 4. The positive values in a set, as determined using a reading table, are added up to give one value for each set. The minimum value for a set is "0", when all reactions are negative, and a maximum of "7", when all the reactions are positive. The combination of these values gives a profile number which is used to find the identity of the culture from a Profile Index Book or an online database.

The API RapiD 20E is designed to identify Enterobacteriaceae in 4hrs (www.biomerieux-usa.com/support/technlibrary/api/index.asp). The system is similar to the API 20E and consists of 20 microtubes that contain substrates for the demonstration of enzymatic activity or fermentation of carbohydrates. The 7-digit profile number that is compiled from test reactions is entered into the APILAB software. The 3.0 software version has a database containing 26 genera and 65 species. Identifications can also be made using the Analytical Profile Index book (www.biomerieux-usa.com/support/technlibrary/api/index.asp).

The Crystal Enteric/ Non-fermenter (E/NF) kit was designed for the identification of Enterobacteriaceae and more commonly isolated glucose-fermenting and non-fermenting Gram-negative bacilli (O'Hara, 2005). The plastic panels consists of 30 tests for the fermentation, oxidation, degradation, or hydrolysis of various substrates. Once the panel is inoculated and snapped together with its lid, it becomes a completely sealed system posing little risk of exposure to the technologist. The software version 4.0 contains 38 genera and 104 species (O'Hara, 2005).

The Enteric Pathogen Screen (EPS) from bioMerieux,is used in conjunction with the Vitek Legacy instrument as a screen for isolates of the common oxidase-negative enteric pathogens (O'Hara, 2005). The card is designed to be presumptive only and any identifications must be confirmed with a GN+ card and serology where appropriate. The cards are self-containing and each card contains 10 substrates. Incubations are carried out in the instrument and reports are generated automatically at the end of the cycle.

The GN2 MicroPlate was designed in 1989 by Biolog Inc for use with any one of the following three microbial identification systems: Omnilog ID, Microlog Micro Station and the MicroLog 1 and 2. The product is based on the exchange of electrons produced during an organism's respiration, leading to a subsequent tetrazolium-based color change from colorless to purple. The GN database version 6.01 contains identification patterns for 526 species that encompass not only Enterobacteriaceae but many other Gram-negative non-fermenters and fastidious organisms.

Biotyping of *Klebsiella* species is not suitable for epidemiological studies because of the large number of reactions and the often long times of incubation (Podschun and Ullman, 1998).

3. Methodology

3.1 Collection, Transportation and Storage of Food Samples

3.1.1 Dried Vegetables

Samples of indigenous dried cow pea leaves (*Vignia unguiculata*) locally known as "Morogo wa dinawa", were purchased from vendors operating in different malls in Gaborone. A total of 22 samples were purchased from BBS Mall, Bus Rank and Main Mall. Care was taken to sample one site per week and sampling each station within a given site. This minimized the chances of resampling the same batch of the vegetables which would give biased results. Each sample collected was assigned a code which was used to label and uniquely identify it. The samples were carried back to the laboratory in their original packages and were stored in a cabinet that excludes light and moisture until the isolation of *K. pneumoniae* could be done. This was done within a week of samples being brought to the laboratory.

3.1.2 Moretlwa Fruits

Samples of the dried wild fruit locally known as "*Moretlwa*", were purchased from vendors operating in different malls in Gaborone. A total of 29 samples were purchased from BBS Mall, Bus Rank and Main Mall. Care was taken to sample one site per week and sampling each station within a given site thus minimizing the chances of resampling the same batch of the fruit, which would give biased results. Each sample collected was assigned a code which was used to label and uniquely identify it. The samples were carried back to the laboratory in their original packages and were stored in a cabinet that excludes light and moisture until the isolation of *K. pneumoniae* could be done. This was done within a week of the samples being brought to the laboratory.

3.1.2 Morula Fruit

Wild fresh *Morula* fruit was picked from the ground under trees in Gaborone and surrounding areas and placed in clean sterile beakers covered with tin foil. Aseptic technique was observed during sampling to ensure that no new organisms were introduced onto the fruit from the sampler's hands. The samples were assigned a unique identifying code which was used to label the samples before transporting them to the laboratory. Sampling frequency was designed to avoid sampling the same tree in quick succession thereby minimizing the chances of sampling the same batch of fruit falling from the tree. The fruit collected was ripe for consumption (the outer skin color was be rich yellow but the fruit still firm when pressed lightly). The samples were carried back to the laboratory in a cooler box on the same day and stored in a refrigerator until isolation of *K. pneumoniae* could be done. This was within two days of sampling.

3.2 Isolation, Storage and Maintenance of Presumptive *K. pneumoniae* from Sources

3.2.1 Dried Vegetables

A weighed portion (25g) of the dried vegetable sample was placed in a sterile stomacher bag. A 100ml of Lauria Broth (LB) was added to the sample which was then placed in a stomacher and homogenized for 5 minutes at moderate speed. The mixture was then transferred aseptically to a sterile 250ml Erlemeyer flask stoppered with a cotton wool plug covered with tin foil. This was placed in an incubator set at 37^{o}C overnight without agitation. After the overnight incubation, the

culture was shaken briefly to uniformly distribute the mixed culture growing in the LB. An inoculum was then streaked out on duplicate plates of Eosin Methylene Blue Agar (EMBA) and incubated overnight at 37^{o}C. After incubation, presumptive *K. pneumonia* colonies, when present, grew to give the characteristic shiny mucoid colonies which were slimy and stringy when touched by an inoculating loop. Representative colonies (showing the typical morphological and phenotypic characteristics of *Klebsiella*) were picked and streaked onto fresh EMBA to multiply the pure culture. The pure culture of the presumptive *K. pneumoniae* was then stored at 4^{o}C on the EMBA until their identity could be confirmed. This was done within a week of the isolation.

3.2.2 Morula fruit

A weighed portion (100g) of the fresh Morula sample was placed in a sterile 500ml beaker and a 200ml of LB was added before closing the beaker with tin foil and incubating at 37^{o}C overnight without agitation. After the overnight incubation, the culture was shaken briefly to uniformly distribute the mixed culture growing in the LB. An inoculum was then streaked out on duplicate plates of Eosin Methylene Blue Agar (EMBA) and incubated overnight at 37^{o}C. After in incubation, presumptive *K. pneumoniae* colonies, when present, grew to give the characteristic shiny mucoid colonies which were slimy and stringy when touched by an inoculating loop. Representative colonies showing the typical morphological and phenotypic characteristics of *Klebsiella* were picked and streaked onto fresh EMBA to multiply the pure culture. The pure culture of the presumptive *K. pneumoniae* was then stored at 4^{o}C on the EMBA until their identity could be confirmed. This was done within a week of the isolation.

3.2.3 Moretlwa

A weighed portion (25g) of the dried fruit sample was placed in a sterile stomacher bag. A 100ml of Lauria Broth (LB) was added to the sample which was then placed in a stomacher and homogenized for 5 minutes at a moderate speed. The mixture was then transferred aseptically to a sterile 250ml Erlemeyer flask stoppered with a cotton wool plug covered with tin foil. This was placed in an incubator set at 37^{o}C overnight without agitation. After the overnight incubation, the culture was shaken briefly to uniformly distribute the mixed culture growing in the LB. An inoculum was then streaked out on duplicate plates of Eosin Methylene Blue Agar (EMBA) and

incubated overnight at 37^{o}C. After in incubation, presumptive *K. pneumonia* colonies, when present, grew to give the characteristic shiny mucoid colonies which were slimy and stringy when touched by an inoculating loop. Representative colonies showing the typical morphological and phenotypic characteristics of *Klebsiella* were picked and streaked onto fresh EMBA to multiply the pure culture. The pure culture of the presumptive *K. pneumoniae* was then stored at 4^{o}C on the EMBA until their identity could be confirmed. This was done within a week of the isolation.

3.3 Identification of Presumptive *K. pneumoniae*

The identification of the presumptive *K. pneumoniae* isolates was done using API 20E and confirmed using the Vitek 2 (bioMerieux) machine.

3.3.1. API20E

The presumptive *K. pneumoniae* isolates on EMBA were transferred to fresh LB, incubated at 37^{o}C overnight and then streaked onto Trypticase Soy Agar (TSA). The TSA plates were then incubated overnight at 37^{o}C. After which the API 20E and Vitek tests where performed.

Gram Stain

The Gram stain was performed on each presumptive *K. pneumoniae* isolate. If the culture was found to be Gram positive, no further tests were done on it and it was discarded. If the culture was found to be Gram negative, the oxidase test was performed.

Inoculating the API 20E Strip

For each presumptive *K. pneumoniae* isolate (gram negative colonies showing typical K. pneumoniae phenotypic characteristics), an incubation box (tray and lid) was prepared and inoculated as outlined in the manufacturer's manual. Each inoculated box was incubated at 37^{o}C for 18-24hrs. After incubation, the results were read and interpreted as outlined in the API 20E instruction booklet. The identification was then done using the Analytical Profile Index Book to translate the numerical profile into and identification of the gram negative bacteria.

3.3.2 Vitek 2 K. pneumoniae Identity confirmation.

The Vitek 2 Machine (bioMerieux) was used to confirm the identities of *K. pneumoniae* isolates. Each isolate identified as *K. pneumoniae* by the API 20E method was subjected to identity confirmation by the Vitek 2 Machine. Dilutions of each of the isolates to be identified were prepared from representative colonies to give a McFarland value of 0.5 on the Densichek equipment. The tube containing the appropriate dilution of the isolates were placed onto a cassette which was then placed on the Smart Station. The sample identity information and the Gram Negative (GN) Card information were then captured into the cassette chip using the Smart Station device. The loaded cassette was then placed into the Vitek 2 Machine for the culture identification. The Machine automatically inoculates the GN cards using the diluted sample, incubates the inoculated GN cards, reads and interprets the colour changes due to the biochemical reactions and gives an identity output within 24hrs.

3.4 Characterization of *K. pneumoniae* Isolates

3.4.1 Determination of Antibiotic Susceptibility of K. pneumoniae Isolates

Antibiotic susceptibility of the isolates was tested by the Kirby-Bauer Disk Diffusion Susceptibility test described below:

Inoculating the MH Agar Plates

About 100ul of prepared bacterial suspension was spotted onto a Muellar-Hinton (MH) agar plate and then evenly distributed on the agar surface using the spread-platting technique. The inoculated MH agar plate was left to dry for about 15 minutes before placing the antibiotic discs.

Placement of the Antibiotic Disks and Incubation

The inoculated MH agar plates were first placed on a prepared template to aid proper placement and separation of the antimicrobial disks. Sterilized forceps were used to place a single antimicrobial disk was placed on the inoculated MH agar plate aligned to one of the dots on the template. This was repeated for each of the seven antibiotics used in this study (see annexure A):

Precautions:- Care was taken to minimize exposure of the inoculated MH agar plates to atmospheric contamination by only partially lifting the petri dish lid to place each antibiotic disk.

Other precautions taken included: avoiding placing disks towards the plate edges, ensuring a 24mm centre to center distance between adjacent disks and ensuring that there was perfect contact between disks and agar surface. Once all antibiotic disks were in place, the MH agar plates were placed in an incubator set at 37^{O}C for 16 to 18 hours.

Reading Results and Interpretation

After incubation, the plates were inspected for presence or absence zones of clearance around the antibiotic disks. Where zones of clearance were present, their diameters were measured using a millimeter ruler with the unaided eye. Readings were rounded up to the nearest millimeter. In cases were a clearance zone extends to the very edge of the plate, the size of the radius of the clearance zone was measured on the side away from the edge and then multiplied by 2 to get the diameter. Were there was no zone of clearance around an antibiotic disk, the diameter was recorded as 0 (zero).

Results

Table 1. *K. pneumonia*e API20E biotype profile frequencies

API20E Profile	Identification confidence level (%)	Frequency	% Frequency
1214773	93.9	5	5.4
5214773	97.4	36	39.1
5215773	97.7	41	44.6
1215773	98.1	4	4.3
4215773	97.7	2	2.2
5015773	97.8	2	2.2
5215573	97.4	2	2.2
Total		**92**	**100**

The most frequent API20E biotype profile encountered was 5215773 at 44.6% and the least encountered API20E biotype profiles were 5215573, 5015773 and 4215773 all of which occurred at a frequency of 2.2%.

Table 2. *K. pneumonia*e isolation rates from food sources

Food Source	**# Of Samples Analysed**	**# Of Samples Positive for *K. pneumoniae***	***K. pneumonia* Isolation Rate (%)**
Morogo wa dinawa	22	19	86.4
Morula fruit	52	48	92.3
Moretlwa fruit	29	25	86.2
TOTAL	**103**	**92**	**89.3**

The isolation rate was highest for *morula* fruit at 92.3% and least for *moretlwa* at 86.2. The overall isolation rate was 89.3%.

Table 3. Occurrence rates of encapsulated *K. pneumoniae*

Food Source	**# *K. pneumoniae* isolates obtained**	**# of encapsulated K. pneumoniae isolates**	**Occurrence rate of encapsulated *K. pneumoniae* (%)**
Morogo wa dinawa	19	10	52.6
Morula	48	15	31.3
Moretlwa	25	10	40
TOTAL	**92**	**35**	**38**

The highest occurrence rate of encapsulated *K. pneumoniae* was in *morogo wa dinawa* at 52.6% and the least was in *morula* at 31.3%. The overall occurrence rate of encapsulated *K. pnuemoniae* in the samples analysed was 38%.

Table 4. Antibiotic susceptibility of *K. pneumoniae* isolates

Antibiotic	Average Size of Zone of Clearance (mm)
Cefotaxime (30ug)	24.4
Ciprofloxacin (1ug)	17.1
Cefoxitin (30ug)	16.1
Nalidixic Acid (30ug)	19.5
Oxacillin (1ug)	0
Cotrimoxazole (25ug)	14.2
Vancomycin (30ug)	0

The largest clearance zone size was 24.4mm for cefotaxime (30ug). The clearance zones for oxacillin (1ug) and vancomycin (30ug) were 0 mm.

Table 5. Frequency of completely resistant *K. pneumoniae* strains per antibiotic

Antibiotic	**# of *K. pneumoniae* strains tested**	**# of completely resistant *K. pneumoniae* strains encountered**	**Proportion of isolates completely resistant to antibiotic (%)**
Cefotaxime (30ug)	92	0	0
Ciprofloxacin (1ug)	92	0	0
Cefoxitin (30ug)	92	0	0
Nalidixic Acid (30ug)	92	0	0
Oxacillin (1ug)	92	92	100
Cotrimoxazole (25ug)	92	21	22.8
Vancomycin (30ug)	92	92	100

None (0%) of the *K. pneumoniae* isolates analysed were completely resistant to cefotaxime (30ug), ciprofloxacin (1ug) and nalidixic acid (30ug). All (100%) of the strains analysed were completely resistant to oxacillin (1ug) and vancomycin (30ug). 22.8% of the isolates were completely resistant to cotrimoxazole (25ug).

Discussion

The study confirmed the occurrence of *K. pneumoniae* on the three foods studied. The isolation rates of the microbe per sample type were 92.3% (*morula fruit*), 86.4% (*morogo wa dinawa*) and 86.2 (*moretlwa*). The overall isolation rate from the three sample types was 89.3%. The higher isolation rate in the *morula* fruit may be attributed to the fact that it was picked straight from the bush and did not undergo any form of microbe reducing processing before it was analysed. On the other hand, both *morogo wa dinawa* and *moretlwa* are subjected to some form of post-harvest processing with the potential to reduce the overall microbial load and *K. pneumoniae* in particular. *Morogo wa dinawa* is boiled, salted and sun dried as part of its preparation for long term storage. Moretlwa is picked fresh from the bush and sun dried before it is sold directly to consumers as a delicacy. These processes may explain the distribution of *K. pneumoniae* on the three foods. The study showed that there is a significant presence of the microbe on the three foods studied therefore there is a potential food safety risk in consuming these foods, particularly by susceptible groups such as immune-compromised individuals.

The API20E identification system used to identify the isolates indicated five different biotype profiles. The most frequent API20E biotype encountered was 5215773 (44.6%) followed by 5214773 (39.1%). The rest of the biotypes (5215573, 5015773 and 4215773) occurred at a frequency of 2.2%. The differences in biotype profiles suggest that there are biochemical differences between the isolates which may point to more subtle differences at the molecular level.

Encapsulated phenotype occurred at an overall rate of 38% in the study. The highest in-sample occurrence rate of the encapsulated phenotype was in *morogo wa dinawa* at 52.6% followed by *moretlwa* at 40% and lastly *morula* at 31.3%. Encapsulated phenotypes are normally associated with higher virulence in *K. pneumoniae.* An interesting observation is that the rate of occurrence of encapsulated phenotype was higher in the food samples which were subjected to some form of processing or more handling. *Morogo wa dinamwa* is handled the most followed by *moretlwa* and lastly *morula*. This would suggest that a considerable quantity of the microbial population on the foods could have been transferred from the food handlers. This has direct public health implications.

Antibiotic susceptibility testing showed that the isolates were most susceptible to 30ug cefotaxime (24.4mm average clearance zone), and all were completely resistant to oxacillin (1ug). For cotrimoxazole (25ug), 22.8% of the isolates were completely resistant. This has implications for antibiotic treatment of community acquired and hospital acquired *K. pneumoniae infections*.

Conclusion

K. pneumoniae is present at significant levels in wild fresh and dried fruits and vegetables consumed in Botswana. There is therefore reason to worry about the possibility of community-acquired Klebsiella pneumonia in susceptible groups emanating from the consumption of wild fruits (fresh and dried) and dried vegetables in Botswana. Further work however needs to be done to fully understand the underlying molecular processes of the disease and how wild *K. pneumoniae* isolates may interact with hospital isolates. Processing, for instance sun drying, boiling, steaming, blanching or cooking reduces microbial load in general and *K. pneumoniae* in particular. These processes need to be properly done to help mitigate the possibility of community-acquired infections from *K. pneumoniae*.

Recommendations

Investigate the effect of different methods of drying on general microbial loads (and *K. pneumoniae* in particular), in dried fruits and vegetables (e.g air-drying, kilning, sun-drying etc)

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Annexures

Annexure A *K. pneumonia*e identification confidence levels using API 20E and Vitek System

Isolate ID	**Identification**		
	AP120E Profile	**Identification Confidence Level (%)**	
		API20E	**VITEK 2**
KP1	1214773	93.9	
KP2	5214773	95	
KP3	5214773	95	
KP4	5214773	95	
KP5	5214773	95	
KP6	5214773	95	
KP7	5214773	95	
KP8	5214773	95	
KP9	5214773	95	
KP10	5214773	95	
KP11	5214773	95	
KP12	5214773	95	
KP13	5214773	95	
KP14	5214773	95	
*KP15	5204773	2 (*82.9 K. terrigena*)	
KP16	5215773	97.7	93
KP17	5215773	97.4	98
KP18	5215773	97.7	99
KP19	5214773	97.4	95
KP20	5215773	97.7	
KP21	5215773	97.7	97
KP22	5215773	97.7	99
KP23	5215773	97.7	99
KP24	5215773	97.7	99
KP25	5215773	97.7	99
KP26	1215773	98.1	99
KP27	4215773	97.7	98
KP28	1214773	93.9	
KP29	5215773	97.7	99
KP30	5215773	97.7	97
KP31	5015773	97.8	
KP32	5215773	97	
KP33	5215573	97.4	
KP34	5215773	97.7	98
KP35	5215773	97.7	94
KP36	5215773	97.7	98
KP37	1215773	98.1	99
KP38	5215773	97.7	93
KP39	5214773	97.4	95

KP40	5214773	97.4	95
KP41			97
KP42			99
KP43	123(1?)5(4?)773	90	
KP44	5214773	95	
KP45	5214773	95	
KP46	5214773	95	
KP47	5214773	95	
KP48	5214773	95	
KP49	5214773	95	
KP50	5214773	95	
KP51	5214773	95	
KP52	5214773	95	
KP53	5214773	95	
KP54	5214773	95	
KP55	5214773	95	
KP56	5214773	95	
KP57	5204773	2 (*82.9 K. terrigena*)	
KP58	5215773	97.7	93
KP59	5215773	97.4	98
KP60	5215773	97.7	99
KP61	5214773	97.4	95
KP62	5215773	97.7	
KP63	5215773	97.7	97
KP64	5215773	97.7	99
KP65	5215773	97.7	99
KP66	5215773	97.7	99
KP67	5215773	97.7	99
KP68	1215773	98.1	99
KP69	4215773	97.7	98
KP70	1214773	93.9	
KP71	5215773	97.7	99
KP72	5215773	97.7	97
KP73	5015773	97.8	
KP74	5215773	97	
KP78	5215573	97.4	
KP79	5215773	97.7	98
KP80	5215773	97.7	94
KP81	5215773	97.7	98
KP82	1215773	98.1	99
KP83	5215773	97.7	93
KP84	5214773	97.4	95
KP85	5214773	97.4	95
KP86			97

KP87			99
KP88	5215773	97.7	98
KP89	5215773	97.7	97
KP90	5215773	97.7	93
KP91	5214773	95	
KP92	5214773	95	

Annexure B Details of Antibiotic Disks Used.

NAME OF ANTIBIOTIC	**ABBREVIATION**	**CONCENTRATION (ug)**	**MANUFACTURER**
Cefotaxime	CTX	30	Mast Group Ltd., Merseyside, UK.
Ciprofloxacin	CIP	1	Mast Group Ltd., Merseyside, UK.
Cefoxitin	FOX	30	Mast Group Ltd., Merseyside, UK.
Nalidixic Acid	NA	30	Mast Group Ltd., Merseyside, UK.
Oxacillin	OX	1	Mast Group Ltd., Merseyside, UK.
Cotrimoxazole	TS	25	Mast Group Ltd., Merseyside, UK.
Vancomycin	VA	30	Mast Group Ltd., Merseyside, UK.