The Potential Role of Door Handles in the Spread of Drug-Resistant Bacteria in Makerere University.

Immaculate Nabawanuka[a,1,2]

[a] Department of Biomolecular Resources of Bio-lab sciences College of Veterinary Medicine Animal Resources and Biosecurity, Makerere University,
Kampala, Uganda

Abstract

Background:[a]
The transmission of diseases caused by pathogenic bacteria is still a threat. One of the potential sources of bacterial diseases is the door handles. This study aimed at isolating, identifying bacteria, determining total bacterial load, and determining antibiotic susceptibility patterns of bacteria obtained from door handles in Makerere university.

Methodology:
A total of 60 samples randomly scattered within the university were swabbed and analyzed for bacterial growth. Samples were inoculated on MacConkey and blood agar and then incubated at 37^{o}c for 24 hours. All sample isolates were sub cultured and identified based on macro and micromorphology, and standard biochemical tests. The establishment of the total bacterial load was done using the standard plate count method. Antibiotic susceptibility testing was done using the disc diffusion method on Muller Hilton agar.

Results:
The following bacterial species and genera were obtained from door handles, *staphylococcus aureus* (30.8%), Coagulase-negative *staphylococcus* (12.0%), *Streptococcus species* (24.2%), *Escherichia coli* (7.7%), *Pseudomonas aeruginosa* (14.3%), bacilli species (11.0%). The study showed that there was a significant difference in the prevalence of bacilli species (p= 0.017) and *E. coli* (p= 0.015) among the study group. The results from total bacterial count indicated that toilet door handles had the highest bacterial load compared to office door handles and classrooms. Antibiotic susceptibility testing of isolates showed that all bacteria were resistant and intermediately resistant to commonly used antibiotics except for *Escherichia coli* that was susceptible to amoxicillin

Conclusion and recommendations:
The study reveals that door handles are a considerable source of pathogenic bacteria thus play a major role in the transmission of diseases caused by such bacteria. Further studies could be done and different study groups could be included for example routinely opened doors and the doors which are not routinely opened.

[a]**email: immienabawanuka@gmail.com**
recieved: 16th/12/2020 accepted: 20th/12/2020
journal of microbiology

1 Background:

Bacteria are the major cause of nosocomial infections counting for 90% of hospital infections (Raka

et al., 2006). Diseases that are caused by bacterial pathogens are of the leading causes of child death around the world, World Health Organization recognizes such diseases as a serious global problem and estimates that each year more than 2.2 million lives lost due to these infections more than from malaria, HIV/AIDS and measles all combined (Burton *et al.,* 2011). In developing countries, it has been investigated that diarrheal diseases are one of the major killers of children and *Escherichia coli* is the most frequent cause of diarrhea in children and adults living in such areas and also among travelers (Qadri *et al.,* 2005). However, no information is documented on the survival of pathogenic bacteria on door handles in Uganda which may be important in the transmission of infectious diseases.

Earlier investigations done by different researchers across the world reported the presence of viable pathogenic bacteria on inanimate surfaces and several studies of the human environment have demonstrated colonization and contamination of objects for example door handles. According to Emeka *et al.*, (2015), in the African Journal of Microbiology Research, in their study to investigate potential pathogenic bacterial contaminants of shared utility devices, the results showed that all samples were contaminated with bacteria, the isolates consisted of *Staphylococcus aureus* (4.02%), *staphylococcus haemolyticus* (18.59%), *staphylococcus* epidermidis(1.10%), other staphylococci (51.76%), *enterococcus feacalis* (2.01%), *Enterococcus* species (1.51%), *Klebsiella pneumonae* (0.5%), *Pseudomonas aeruginosa* (14.03%), *pseudomonas stutzeri* (3.5%), Pseudomonas luteola (10.53%), the results also indicated the presence of multi antibiotic-resistant bacterial strains among shared items and this could be a potential source of infection in the university setting. In a study by Oluduro *et al*., (2011), in the bacterial assessment of electronic hardware user interfaces where also drug susceptibility of isolated bacteria was tested, the frequencies of occurrence of the species were Aerococcusvaridans (9.4%), *Bacillus* species (8.4%), *Enterobacteraerogenes* (4.9%), *Gaffkyatetragena* (2.1%), *Klebsiella pneumonae* (11.1%), micrococcus luteus (10.9%), Moraxella catarrhally (1.6%), *Proteus* species (10.6%), *Pseudomonas aeruginosa* (16.0%), *Staphylococcus aureus* (16.7%), and *Staphylococcus epidermis* (8.2%), It was found out that all interfaces were contaminated and most isolates were resistant to amoxicillin, Augmentin, nitroflantoin, and ceftriaxone while resistance to ciprofloxacin and ofloxacin was the least frequent. Multiple antibiotic resistance was observed in 89.1% of bacterial isolates, with a total of 68 resistance patterns, resistance to three antibiotics being the most frequent (31.9%). Aminu *et al.*, (2015) conducted a study to determine the antibiotic susceptibility pattern of bacteria isolated from fomites in a teaching hospital in Nigeria, the bacteria isolated were *Staphylococcus aureus* (21.7%), *staphylococcus epidermidis* (8.7%), *Streptococcus species* (8.7%), *Bacilli* (13.0%), *E.coli* (26.1%), *pseudomonas* species (8.7%) and *Klebsiella* species (13.0%), the isolated bacteria showed varying susceptibility patterns to the antibiotics used and were all susceptible to Erythromycin and streptomycin.

Previous studies here show that the spread of infectious diseases through hand contact has been an area of concern, where 80% of the infections are spread through hand to hand contacts as well as hands to other surfaces. Door handles have been shown to play a major role in the transmission of pathogenic bacteria that are potential causes of infectious diseases. However, the potential role of door handles in the spread of drug resistant bacteria has not been well documented in Uganda.

2 MATERIALS AND METHODS

Study area and design

This was a cross-sectional study in which door handles were swabbed to obtain samples from various places in Makerere University that included lecturers' offices, toilets, and classrooms of students. The samples were collected and were assigned identification numbers and organized into categories: offices, toilets, and classrooms for analysis.

Study population and sample size determination

The sample size of the study was estimated using the Kish and Lesile (1965) formula as below;

N= Zpq/L2

D

Where; N was the estimated sample size

Z Was the standard deviation at 95% confidence interval

p (estimated prevalence) was the probability to achieve the studied phenomenon

q (1-p) was the compliment of the estimated prevalence

d (desired precision) = margin of error (0.05)

L= the degree of precision which in this case was 1-10% (0.1).

Therefore, the sample size was

N= 1.96*0.12(0.12)/0.12

0.05

N= 60

2.1 Total of 60 samples were collected

The samples for this study were collected at a single point in time from lecturers' offices, students' classrooms, and toilets. There were 20 samples collected for each category respectively.

Sampling and sample collection.

Samples were collected purposively from the different places i.e. Sampling was done using a damp sterile swab by rotating the swab all around the door handle concentrating more at the tip of the door handle that is constantly handled thus getting contaminated with unclean hands, the swab was then returned to its sterile container, containing sterile transport media, the container was labeled with the date of collection, office, classroom or toilet and finally put in the cool box to be transported to the microbiology laboratory at Mengo hospital for further processing and analysis.

Laboratory procedures

Preparation of media.

Media for use was blood agar, MacConkey, Muller Hinton agar, and nutrient agar. Media preparation was done following the manufacturer's instructions found on the media bottles. Appropriate amounts of powder media were weighed then placed in a conical flask and the appropriate amount of distilled water was added to dissolve the powder. After which the media was sterilized using an autoclave at 121^{o}C for 15 minutes. Media was then allowed to cool before it was poured into the Petri dishes arranged on a level surface.

Gram staining

The differentiation of the organisms to know the gram-positive and gram-negative bacteria was done using the gram staining technique (Baker *et al.*, 1985); with a sterile loop, a colony was picked to make a smear and allowed to dry. The smear will be fixed over a flame by gentle flaming and slide rested onto a staining rack. It was then flooded with 0.5% crystal violet for 1 minute, washed off with water, and replaced with grams' iodine solution for 1 minute. Excess iodine then was washed off and decolorized with 50% acetone spirit for 30 seconds. Decolorizer was then washed off with water then counterstained with 1% carbofuschsin for 1 minute. The counter stain was then washed off, smear blotted to dry, and examined under a microscope with x100 oil immersion objective lens.

Biochemical tests

Catalase test

A colony of the suspected organism was transferred, using a sterile wire loop from the culture to a sterile slide. A drop of hydrogen peroxide solution was added and observed for the reaction. Effervescence or bubbling was observed for positive samples and no bubbling for negative samples.

Coagulase test

Staphylococcus aureus was known to produce coagulase, which can clot plasma into a gel in a tube or agglutinate cocci in the slide. This test was useful in differentiating S.aureus from other coagulase-negative *staphylococci.* Dense suspension of *staphylococci* from culture was made on two ends of a clean glass slide. One pure colony of the suspected organism was added into one drop of plasma on a sterile glass slide using a sterile wire loop. Coagulation of the plasma was observed for coagulase-positive samples while no coagulation was observed for coagulase-negative isolates.

Indole test

This test was based on the ability of *E. coli* to liberate indole from amino tryptophan by liberating the amino group to form indole, pyruvate, and ammonium. A few drops of Kovac's reagent were added to incubated peptone water containing the organism under investigation. The appearance of a pink-colored lining on the surface confirmed a positive reaction (presence of *E. coli*) while retention of the straw color of the reagent on the surface of the peptone indicated the absence of *E. coli.*

Urease test

This test indicated whether or not an organism can utilize citrate (citric acid) as a nutrient. Simmons Citrate agar slants were used for this test. The slants were prepared such that citrate was the only carbon source, thus forcing the organism to use it as a nutrient. The medium also had a pH as an indicator called Bromthymol blue. The slants were inoculated and bacteria that we're able to utilize citrate as a fuel catabolized the citrate in the medium and released an end equal product that was basic (alkaline). The indicator Bromthymol blue is blue above pH 7.6 and green at pH values below

7.6. If citrate was utilized, the medium pH raised and the medium turned from green to blue.

Bacterial isolation and identification.

MacConkey and blood agar were prepared for bacterial inoculation. Samples were inoculated onto MacConkey and blood agar plates using a sterile loop by streaking method. The inoculated plates were incubated at 37^{o}c for 24 to 48 hours before they were subjected to secondary culture and identification. The single colony of grown bacteria was isolated and subcultured on pure solid media. Biochemical tests were conducted according to WHO *et al.,* (2003), and they included catalase, coagulase, oxidase, triple sugar iron. Identification of bacterial isolates was done using macro morphological characteristics of bacterial colonies, micromorphology of the bacteria on the gram stain, and biochemical characteristics of the bacterial isolates.

Total bacterial count from the samples.

Bacteria were counted using the standard plate count method, samples were diluted in normal saline followed by a 10-fold serial dilution with two dilution steps per sample, one milliliter of the last dilution step was added to a sterile agar plate and 15 mls of agar pre-cooled to 45oc was poured into each plate and swirled gently to mix. After agar solidification, the plates were inverted and incubated for 72 hours at 30^{o}c. Counting the colonies was made easy by dividing the plate into four equal parts and all the plates contained between 30 to 300 colonies thus easy counting. Total bacterial load in terms of CFU/ml was calculated from the formula below.

CFU/ml = Number of colonies counted x dilution factor

Volume of the culture plate.

Antibiotic susceptibility testing

The Kirby Bauer disc diffusion method was used. The preserved bacterial isolates were thawed and allowed to come to room temperature. A sterile wire loop was used to obtain a loop full of the isolate and then streaked over Muller Hinton agar evenly to form a mattified surface. Antibiotic discs were then placed onto the plate equally spaced out using sterile forceps, six discs that included amoxicillin, gentamicin, streptomycin, cefuroxime, cotrimoxazole, and ampicillin were placed on a single plate. After this, the plates were incubated at 37oc for 18 hours. The zones of inhibition were measured using a ruler to the nearest milliliter and compared to the standard provided by the National Committee for Clinical Laboratories (2003). The zones were interpreted as susceptible, intermediate, or resistant using Reference E. coli strain ATCC 25922 as a quality control test.

3 Data analysis and management.

Data were recorded in the laboratory record sheets then entered into Microsoft Excel and analyzed. Comparison between the microbial status of the different door types i.e. offices, classrooms, and toilets were done using a chi-square comparison test with a set significant value of $p<0.05$. Results were computed in SPSS version 20 for analysis and presented in the table form.

4 RESULTS.

Bacterial isolation and identification

Out of the 60 samples collected from the door handles, 49 (86.0%) showed bacterial growth, the predominant contaminated door handles were the toilet door handles with 95% positive samples, followed by classrooms (85%) and lastly, office door handles with 70% positive samples.

The bacterial isolates obtained from the door handles were *staphylococcus aureus* (31%), coagulase-negative *staphylococci* (12.0%), *streptococcus* (24%), *Escherichia coli* (8%), *pseudomonas aureginosa* (14%), and bacilli species (11%). The most frequently isolated bacteria were *Staphylococcus aureus* with 28(31%) of the isolates. Among the study groups, classroom door handles had the highest (39%) prevalence of *staphylococcus aureus* followed by toilets (36%) and lastly offices (25%). Results showed no significant difference (p=0.695) among the *staphylococcus aureus* isolated from the study groups. The second most isolated bacteria were the *streptococcus* 22 (24%) found on 50%,32%,18% door handles of toilets, classrooms, and offices respectively. The study found no significant difference (p= 0.201) in the prevalence of *streptococcus* on the door handles of the study groups. *Pseudomonas aeruginosa* 13(14%), was isolated from 46%,38%, and 15% of door handles of offices, classrooms, and toilets respectively. Results showed no significant difference (p= 0.17) in the prevalence of *Pseudomonas aeruginosa* among door handles of the study groups. Coagulase-negative *staphylococcus* 11(12%) were isolated from 82%,18% door handles of toilets and classrooms respectively, none

Table 1. The incidence of positive samples of the door handles

Sources	Total samples collected	No of positives	% of positive samples
Offices	**20**	**14**	**70**
Classrooms	**20**	**16**	**85**
Toilets	**20**	**19**	**95**
Total	**60**	**49**	**86**

was isolated from offices. The study showed no significant difference (p= 0.167) in the prevalence of CoNS door handles of the study groups. Bacilli species (11%) were isolated from 20%, 20%, and 60% door handles of offices, classrooms, and toilets respectively. There was a significant difference (p= 0.017) in the prevalence of bacilli species on door handles of toilets as compared to offices and classrooms. *E. coli* (8%) was isolated from 100% door handles of toilets, and 0% for offices and classrooms. There was a significant difference (p= 0.015) in the prevalence of *E. coli* on door handles of toilets as compared to classrooms and offices as shown in table 2.

Key; CoNS – coagulase-negative staphylococcus, spp – species, *E.coli – Escherichia coli*

Total bacterial load on the door handles.

The bacterial count was done for all the positive samples and the mean CFU/Ml for each source was obtained, this was the total bacterial load in terms of CFU/ML for each source. The total bacterial load for each source was as follows; offices had a total bacterial load of 4.0x10^6 CFU/ml, classrooms had 8.6x10^6 CFU/ml, and lastly, toilets had 2.0x10^7CFU/ml., as shown in table 3

Key; CFU/ml – colony forming units per millilitre

Antibiotic susceptibility testing.

The susceptibility patterns of isolates revealed varying degrees of resistance to the six commonly used antibiotics as shown in the table;

Coagulase-negative *staphylococcus* was resistant to all the antibiotics except for amoxicillin where 64% was susceptible, *staphylococcus aureus* showed intermediate susceptibility to amoxicillin (61%), ampicillin(50%) and it was resistant to the rest of the antibiotics, streptomycin was resistant to all the antibiotics apart from amoxicillin(68%) where it showed intermediate susceptibility, *Eshcerichia coli* was resistant to all except for amoxicillin(86%) were it was susceptible, *pseudomonas aeruginosa* showed intermediate susceptibility to amoxicillin(54%), gentamicin(54%) and was resistant to the rest, bacilli was resistant to all except for cefuroxime(60%) and cotrimoxazole(60%) where it showed intermediate susceptibility

Key; Amc – Amoxicillin, CN – Gentamicin, S- streptomycin, Cep – Cefuroxime, Cot – Cotrimoxazole, Amp- Ampicillin

5 DISCUSSION.

Previous studies have shown the potential role of door handles in the transmission of infections for example studies by Nworie *et al.,* (2012) revealed different pathogenic bacteria that contaminate door handles. The aim of this study, therefore, was to isolate, identify, establishing the bacterial load on door handles as well as determining the antibiotic susceptibility patterns of the bacterial isolates

Out of the 60 samples processed, 49 (86.0%) showed bacterial contamination, this is in agreement with the reports of some researchers who observed 86% positive samples, a study on door handles carried out by Nworie *et al.,* (2012) in Nigeria observed 156 (86.7%) bacterial contamination, this was slightly lower than that from reports of Otter and French who observed 95% positive cultures in London. In this study, the level of contamination was higher in toilets, this is in agreement with Scott *et al.,* (1982) who stated that toilets are associated with higher levels of microbial contamination, next to toilets were classrooms and lastly were the lecturers' offices with the lowest numbers of positive samples. This study is in agreement with the findings of and Nworie *et al.*, (2012) who reported that the levels of contamination vary depending on traffic exposure and environment, lecturers' offices are associated with low levels of contamination because they observe maximum hygiene and sanitation as compared to the students, classrooms are associated with low levels of contamination as compared to toilets because they are not frequently touched

Table 2. Percentage of bacterial isolates from different sources.

	s. aureus	CoNS	Streptococcus	E.coli	Pseudomonas aeruginosa	Bacilli spp
Offices	7(25%)	0	4(18%)	0	6(46%)	2(20%)
classrooms	11(39%)	2(18%)	7(32%)	0	5(38%)	2(20%)
Toilets	10(36%)	9(82%)	11(50%)	7(100%)	2(15%)	6(60%)
Total	28(31%)	11(12%)	22(24%)	7(8%)	13(14%)	10(11%)
P value	0.695	0.167	0.201	0.015	0.17	0.017

Table 3. Table 3; The total bacterial load on the door handles.

Sources	Total bacterial load (CFU/ml)
Offices	$4.0x10^6$
Classrooms	$8.6x10^6$
Toilets	$2.0x10^7$

Table 4. The antibiotic susceptibility patterns of the isolated bacteria Isolates

Isolates	CoNS n=11	S.aureus n=28	Streptomycin n=22	E.coli n=7	p.aeruginosa n=13	Bacilli ssp n=10
Amc	7(64%) (I)	17(61%) (R)	15(68%) (I)	6(86%) (S)	7(54%) (I)	4(40%) (R)
CN	5(45%) (R)	0(R)	6(27%) (R)	3(43%) (R)	7(54%) (I)	3(30%) (R)
S	3(27%) (R)	11(39%) (R)	9(41%) (R)	2(29%) (R)	4(31%) (R)	6(60%) (I)
Cep	2(18%) (R)	10(36%) (R)	6(27%) (R)	1(14%) (R)	0(R)	6(60%) (I)
Cot	2(18%) (R)	0(R)	0(R)	1(14%) (R)	0(R)	0(R)
Amp	1(9%) (R)	14(50%) (I)	0(R)	3(43%) (R)	0(R)	3(30%) (R)

In this study the most frequently isolated bacteria were *Staphylococcus aureus* 28 (31%) which would be because it is the major component of the normal flora of the skin and nostrils which probably explains its high prevalence as a contaminant as it can easily be discharged by several human activities like sweating. The bacteria isolated from door handles during this study were, *Staphylococcus aureus,* Coagulase-negative *staphylococcus*, *Streptococcus, Pseudomonas aeruginosa*, *Escherichia coli,* and Bacilli species.

Possible diseases that can be caused by the isolated bacteria include Foodborne diseases (*S. aureus* and *E. coli*), urinary tract infections (*E. coli and P. aeruginosa*) Total bacterial load was done from samples collected from the three sources in Makerere university, the results of this study showed that door handles of toilets that are routinely used by many people had the highest bacterial load compared to class rooms and lecturers offices, this observation could be as a result of poor sanitary conditions before and after use of toilets for example not washing hands thoroughly with a disinfectant.

6 Conclusion and Recommendations

7 Conclusion.

The study reveals that door handles are a considerable source of pathogenic bacteria thus play a major role in the transmission of diseases caused by such bacteria. This study has shown that 86% of the door handles from the three study groups

(toilets, offices, classrooms) had bacterial contamination. Toilet door handles are the most contaminated of the three categories with all the bacteria isolated compared to other study groups, the most common bacteria isolated is *staphylococcus aureus*, followed by *streptococcus, pseudomonas aeruginosa*, coagulase-negative *staphylococcus*, bacilli species, and Lastly *E. coli*. Antibiotic resistance patterns were observed for all the commonly used antibiotics except for amoxicillin that was susceptible to *E. coli*, thus good sanitary measures should highly be observed for example routine handwashing with disinfectant not only after use of toilets, routine disinfection of door handles, as they are the first line of protection from transmission of bacterial infections.

Recommendation

Further studies could be done and different study groups could be included for example routinely opened doors and the doors which are not routinely opened probably by the use of questionnaires. Studies should also be carried on other surfaces for example parts of the doors other than the handles, taps, chairs, toilet seats to investigate the potential role of the door handles in the spread of drug-resistant bacteria in Makerere university.

Acknowledgment.

I thank the Almighty God for the gift of wisdom and understanding that have helped me so much to produce this work, I also thank God for the gift of life and his provision in all aspects.

I thank my supervisor, Dr. Kato Charles Drago, for allowing me to supervise and guide me during the production of this work, may the Almighty God bless you. I thank my parents for their endless support towards my academics, may God reward you abundantly.

I thank my friends, Damien, Esther, and Diana for their courage and academic support, may God reward you abundantly.

Abbreviations and Acronyms.

Coli : *Escherichia coli*
et al : and others
mg : milligram
mg/l : milligram per liter
Staph.aureus : *Staphylococcus aureus*
WHO : World Health Organization.
i.e. : That is to say
e.g. : For example
MRSA : Methicillin-resistant *staphylococcus aureus*
VRSA : Vancomycin-resistant Staphylococcus aureus
GISA : Glycopeptides intermediate *staphylococcus aureus.*
UTIs : Urinary tract infections
Mls : milliliters

A References:

1) Adger-Emeka,L. I., Al-Sultan, A. A., Al-Dehailan, H. S., Al-Humini, N. K., Al- Najja, F. A., Al-Farhan, H. M. (2015). Potential pathogenic bacterial contaminants of shared utility devices in a university setting at Al-Hofuf, Saudi Arabia. African Journal of Microbiology Research, 9(41), 2139-2144. https://doi.org/10.5897/AJMR2015.7577

2) Aminu M., Usman S. H. and Usman M. A. (2014). Characterization and determination of antibiotic susceptibility pattern of bacteria isolated from some fomites in a teaching hospital in northern Nigeria. Afr J Microbiol Research. https://doi.org/10.5897/AJMR2013.6512

3) Burton M., Emma C., Peter D., Gaby J., Val C. and Wolf-Peter S. (2011). The effect of hand washing with water or soap on bacterial contamination hands. Int. J. Environ. Res. Public health.

4) Cheesbrough M. (2006). District laboratory practice in Tropical countries, Part 2, Cambridge university press, United Kingdom. https://doi.org/10.1017/CBO9780511543470 PMCid:PMC2870630

5) Nworie .A., Ayeni .J. A., Eze .U. A., Azi .S. O.(2012), "Bacterial contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria: A public health threat." Wilolud Journals. 6(1):7-11.

6) Oluduro. A. O, Ubani .E. K., Ofoezie. I. E, (2011) "Bacterial Assessment of Electronic Hardware User Interfaces in Ile-Ife, Nigeria." Journal of basic and applied Pharmacology. https://rcfba.fcfar.unesp.br/index.php/ojs/article/view/325

7) Qadri, F., Svennerholm, A. M., Faruque, A. S., & Sack, R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clinical microbiology reviews, 18(3), 465-483. https://doi.org/10.1128/CMR.18.3.465-483.2005. https://doi.org/10.1128/CMR.18.3.465-483.2005 PMid:16020685 PMCid:PMC1195967

8) Raka L, Zoutman D, Mulliqi G, Krasniqi S, Dedushaj I, Raka N, et al. Prevalence of nosocomial infections in high-risk units in the university clinical center of Kosova. Infect Control. 2006;27(04):421-3. https://doi.org/10.1086/503387 PMid:16622824

9) Scott, E., Bloomfield, S., & Barlow, C. (1982). An Investigation of Microbial Contamination in the Home. The Journal of Hygiene, 89(2), 279-293. Retrieved December 17, 2020, from http://www.jstor.org/stable/3863039 https://doi.org/10.1017/S0022172400070819 PMid:7130703 PMCid:PMC2134222