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Hygienic practices and prevalence of *Klebsiella pneumoniae* among butchers in a beef slaughterhouse: A comprehensive study

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Abstract
This study presents a comprehensive investigation into the prevalence of *Klebsiella pneumoniae* among 55 beef butchers in a Pondicherry Municipality slaughterhouse. Combining meticulous bacteriological analysis of hand swab samples with advanced statistical methods, the study explores the nuanced relationship between hygienic practices and *K. pneumoniae* prevalence. Logistic regression analysis reveals a significant negative association between proper hand washing and the likelihood of *K. pneumoniae* infection, offering a statistical basis for hygiene interventions. While equipment cleaning and personal protective equipment usage showed no significant associations, the overall prevalence of *K. pneumoniae* was found to be 10.91%. These statistical insights underscore the urgent need for targeted education on hygienic practices in slaughterhouse settings. The study provides valuable quantitative data, shedding light on the statistical patterns governing *K. pneumoniae* dynamics and hygiene practices among beef butchers.

Keywords: *Klebsiella pneumoniae*, statistical analysis, logistic regression, hygiene practices, beef slaughterhouse

Introduction

Klebsiella pneumoniae, belonging to the *Enterobacteriaceae* family, is pervasive in the natural environment and colonizes the gastrointestinal tracts of healthy humans and animals. They are gram-negative, non-motile, capsulated bacteria that are facultative anaerobic. (Wareth and Neubaeur, 2021) [8]. *K. pneumoniae* is a colonizer of livestock, a contaminant of retail meats and vegetables, and a cause of extra-intestinal infections in humans. It is the leading cause of ventilator-associated pneumonia, neonatal sepsis, and hospital-acquired pneumonia. It also causes community-acquired pyogenic liver abscess, meningitis, endophthalmitis, cellulitis, and non-alcoholic fatty liver disease (Ikuta *et al*., 2022; Yuan *et al.,* 2019; Wyres *et al*., 2020) [3, 10, 9]. There is a growing concern over the increasing number of *K. pneumoniae* isolates resistant to many antibiotics. The use of antimicrobials in livestock has contributed to the emergence and spread of antimicrobial-resistant bacteria, including *K. pneumoniae*. This issue is particularly pronounced in developing nations, where antimicrobial use is widespread and unregulated, exacerbating the problem at a faster rate (Montso *et al*., 2019) [5]. In the absence of accurate statistics, evidence suggests a significant burden of disease caused by *K. pneumoniae* infection. Despite a few studies reporting on the presence of *K. pneumoniae* strains in humans and their antimicrobial susceptibility, there is a lack of investigations into the extent of *K. pneumoniae* contamination in abattoir environments.

Materials and Methods

The Pondicherry Municipality operates a single authorized slaughterhouse exclusively designated for beef. Within this facility, a workforce comprising 55 skilled butchers is engaged in the meat production process. A meticulous investigation was conducted by collecting 55 hand swabs from these butchers, and subsequently, a comprehensive screening for the presence of *K. pneumoniae* was undertaken using established bacteriological methodologies. The samples were inoculated onto Luria Bertani broth and streaked onto HiCrome Klebsiella selective agar after incubation for 18-24 h at 37 C. appearance of purple magenta coloured smooth mucoid colonies were considered positive for *K. pneumoniae*. The study also included a thorough examination of the hygienic practices adhered to by the butchers, including

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protocols for hand and equipment washing, as well as the utilization of protective gear such as gloves and aprons. In addition to hygienic procedures, demographic information pertaining to the butchers was systematically documented, encompassing details such as age, gender, professional experience, and marital status. A logistic regression analysis was carried using R-studio software version 1.4.1564 for the prevalence of *K. pneumoniae* and the hygienic practices followed by the butchers to find out the factors which affect the prevalence of the organism. Institutional ethical committee approval was obtained from Indira Gandhi Medical College and Research Institute, Puducherry (No. 451/IEC-36/IGMC&RI/PP12-2022 dated 01.12.2022) for conducting the study on butchers

Results and Discussion

A total of 06 (10.91%) *K. pneumoniae* were isolated from hand swab samples. The colonies are shown in Fig. 1. In the present study the prevalence of *K. pneumoniae* was found to be 10.91% in the hand swab samples of the butchers. Our study results were in agreement with the findings of Silago *et al*. (2021) [7] who reported a prevalence rate of 9.1% for *K. pneumoniae* in hand swab samples of nurses and mothers in a Tanzanian hospital. However, our findings were in sharp contrast to those reported by Gwida *et al*. (2014) [2] in Egypt, who found a prevalence rate of 24% for *K. pneumoniae* in hand swab samples of abattoir workers, and Josy *et al*. (2018) [4] in Chennai, who found a prevalence rate of 15.83% for *K. pneumoniae* in hand swab samples of animal handlers. The finding that 6 out of 55 hand swabs from butchers were positive for *K. pneumoniae* is of significance as it highlights the potential for the transmission of this bacterium from animals to humans. Butchers who are colonized with *K. pneumoniae* may serve as a potential reservoir for the transmission of *K. pneumoniae* strains to other animals, or to consumers who handle or consume contaminated meat products.

During our observation of the butchers' hygiene practices at the slaughterhouse, we noted that 62% of the butchers properly washed their hands before slaughtering, while only 40% washed their knives before use. Additionally, 44% of the butchers wore aprons, but none used gloves during the slaughtering process. Other details of the hygienic practices followed by the butchers are given in figure 2. Our resulta are in accordance with the findings of Mulu and Pal, 2016 [6], who reported 56% of the butchers in an Ethiopian slaughterhouse washed their hands, but are in contrast with the report of 80% butchers washing their equipment and almost all wearing a type of protective gear (hair covering). Further education on hygienic practices must be provided for the butchers is needed.

The butchers in the Pondicherry Municipality-approved beef slaughterhouse were all men. Most of them, around 55%, finished their schooling, while a smaller percentage, 3.6%, had a college education. The majority, about 62.3%, were married, while 32.7% were unmarried. In terms of age, most butchers fell between 25 and 50 years old, with the average age being 38.5 years. Regarding their professional background, 53% of the butchers had gained 6-10 years of experience in the slaughterhouse. Socio-demographic details of the butchers are given in Table 1. The results of our study on butchers' socio-demographic details are in accordance with the study of Berhanu *et al*., 2021 [1] which reported majority of the workers are middle aged and median experience of 6-10 years.

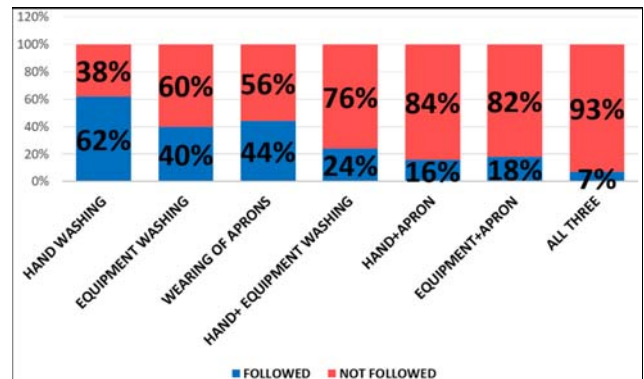**Fig 1:** *K. pneumoniae* on Hicrome Klebsiella selective agar

Fig 2: Hygienic practices followed by the butchers in slaughterhouse

Table 1: Socio-demographic details of the butchers working in Pondicherry Municipality slaughterhouse

Variables	Response	Frequency	Percentage (%)
Age	18-25	05	9.1
	25-50	28	50.9
	>50 years	22	40
Educational status	Illiterate	23	41.8
	School	30	54.6
	Graduate	02	3.6
Marital status	Married	37	67.3
	Unmarried	18	32.7
Religion	Hindu	07	12.7
	Christian	15	27.3
	Muslim	33	60
Experience	1-5 years	05	9.1
	6-10 years	29	52.7
	>10 years	21	38.2

The logistic regression analysis was done using R-studio software. The prevalence of *K. pneumoniae* in butchers and hygienic practices followed by them in the slaughterhouse showed that the estimate for *K. pneumoniae* was -1.3225 (SE = 0.6667, z = -1.984, p = 0.0473*), indicating a significant negative association with hygienic practices. The estimates for hand washing (HA), equipment cleaning (EQ), and personal protective equipment (PPE) were -1.2731 (SE = 0.9284, z = -1.371, p = 0.1703), -0.1332 (SE = 0.9554, z = -

0.139, p = 0.8891), and -0.2595 (SE = 0.9582, z = -0.271, p = 0.7865), respectively, suggesting no significant association with *K. pneumoniae* prevalence.

Table 2: Logistic regression model output for the prevalence of *K. pneumoniae* and hygienic practices in the slaughterhouse

	Estimate	SE	p- value*	Odds ratio
Intercept	-1.3225	0.6667	0.0473[S]	-
HA	-1.2731	0.9284	0.1703	0.28
EQ	-0.1332	0.9554	0.8891	0.875
PPE	-0.2595	0.9582	0.7865	0.771

*- Wald test
[S]- Statistically significant
The percentage reduction in the odds of having *Klebsiella pneumoniae* = (1- odds ratio)* 100

The odds ratio of 0.28 for hand washing practice indicates that butchers who follow hand washing are 72% less likely to have *K. pneumoniae* than butchers who do not follow hand washing. The odds ratio of 0.875 for equipment washing practice indicates that the butchers who follow equipment washing are 12.5% less likely to have *K. pneumoniae* than butchers who do not follow the practice. The odds ratio of 0.771 indicates that the butchers who follow wearing of aprons are 22.9% less likely to have *K. pneumoniae* than the butchers who do not follow the practice. The logistic regression coefficient plot is given in Fig. 3. Based on the logistic regression model for the prevalence of *K. pneumoniae* in butchers and the hygienic practices followed by them in the slaughterhouse there was a negative association between the intercept and the occurrence of *K. pneumoniae*. We found that there would be significant reduction in the prevalence of *K. pneumoniae* in the butchers, if they follow the hygienic practices properly. The results of this model strengthens the argument on improving the hygienic practices of the butchers in the slaughterhouse.
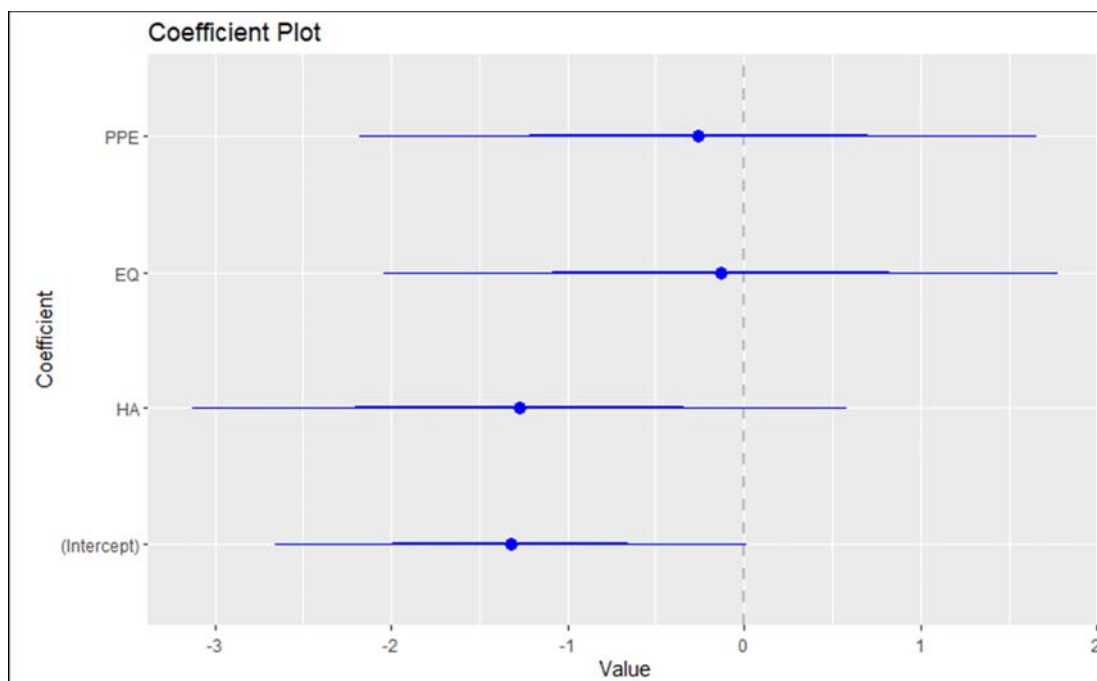

Fig 3: Logistic Regression Coefficient Plot

Conclusion
In conclusion, this study sheds light on the prevalence of *Klebsiella pneumoniae* among butchers in a beef slaughterhouse in Pondicherry Municipality. The findings underscore the significance of hygienic practices in mitigating the transmission of *K. pneumoniae*. A notable 10.91% prevalence of *K. pneumoniae* was observed in hand swab samples, emphasizing the potential for transmission from butchers to both animals and consumers. The logistic regression analysis revealed a significant negative association between hygienic practices and *K. pneumoniae* prevalence, with proper hand washing demonstrating a 72% reduction in likelihood. The study also highlighted areas for improvement, including low rates of equipment cleaning and limited use of personal protective equipment. Enhancing hygiene education for butchers could contribute to a substantial decrease in K. pneumoniae prevalence. These findings underscore the importance of stringent hygienic measures in slaughterhouse environments to minimize the risk of bacterial transmission and ultimately safeguard public health

References
1. Berhanu L, Bedru H, Gume B, Tolosa T, Kassa T, Getaneh A, *et al*. Occurrence, risk factors, and antimicrobial susceptibility test of thermophilic campylobacter species of bovine carcass at municipal abattoir and butcher shops of Jimma Town, Southwest Ethiopia. Infection and Drug Resistance; c2021. p. 3753-3762.
2. Gwida M, Hotzel H, Geue L, Tomaso H. Occurrence of *Enterobacteriaceae* in raw meat and in human samples from Egyptian retail sellers. International scholarly research notices; c2014.
3. Ikuta KS, Swetschinski LR, Aguilar GR, Sharara F, Mestrovic T, Gray AP, *et al*. Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. The Lancet. 2022;400(10369):2221-2248.
4. Josy A, Gunaseelan L, Porteen K, Sriram P, Anusha SA, Balaji P. Antimicrobial Resistance Pattern of *Klebsiella* spp. Isolated from Animal Handlers. Journal of Animal

Research. 2018;8(4):727-730.

5. Montso KP, Dlamini SB, Kumar A, Ateba CN. Antimicrobial resistance factors of extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Cattle Farms and Raw Beef in North-West Province, South Africa. BioMed research international; c2019.

6. Mulu S, Pal M. Studies on the prevalence, risk factors, public health implications and antibiogram of *Listeria monocytogenes* in sheep meat collected from municipal abattoir and butcher shops in Addis Ababa. Journal of Foodborne and Zoonotic Diseases. 2016;4(1):1-14.

7. Silago V, Kovacs D, Samson H, Seni J, Matthews L, Oravcová K, *et al*. Existence of multiple ESBL genes among phenotypically confirmed ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* concurrently isolated from clinical, colonization and contamination samples from neonatal units at Bugando Medical Center, Mwanza, Tanzania. *Antibiotics*. 2021;10(5):476.

8. Wareth G, Neubauer H. The Animal-foods-environment interface of *Klebsiella pneumoniae* in Germany: an observational study on pathogenicity, resistance development and the current situation. Veterinary Research. 2021;52(1):1-14.

9. Wyres KL, Wick RR, Judd LM, Froumine R, Tokolyi A, Gorrie CL, *et al*. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of *Klebsiella pneumoniae*. PLoS genetics. 2018;15(4):e1008114.

10. Yuan J, Chen C, Cui J, Lu J, Yan C, Wei X, *et al*. Fatty liver disease caused by high-alcohol-producing *Klebsiella pneumoniae*. Cell metabolism. 2019;30(4):675-688.