May 28, 2024

“Treated and untreated cows housed side by side in tie-stalls and their respective risk of harboring E. coli resistant to antimicrobials”

DOI
dx.doi.org/10.17504/protocols.io.j8nlk8ojxl5r/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.j8nlk8ojxl5r/v1

Protocol Citation: Belinda Köchle, Véronique Bernier Gosselin, Heike Kaspar, Jens Becker 2024. “Treated and untreated cows housed side by side in tie-stalls and their respective risk of harboring E. coli resistant to antimicrobials”. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.j8nlk8ojxl5r/v1**

Protocol status: Working
We use this protocol and it's working

Created: May 28, 2024

Last Modified: May 28, 2024

Protocol Integer ID: 100701

Funders Acknowledgement:
Fondation Sur-la-Croix (Basel, Switzerland)
Vontobel foundation (Zurich, Switzerland)

Abstract

Parenteral antimicrobial
treatment results in the excretion of antimicrobial-resistant bacteria. Dairy
cows are commonly housed side by side in tie-stalls and often receive
antimicrobial treatment. However, studies investigating treated cows as source
of colonization of neighboring cows with resistant bacteria are scarce.
Antimicrobial resistance (AMR) in cows (treated and untreated) in tie-stalls was
investigated to assess their respective risks of carrying resistant bacteria.
Furthermore, we analyzed associations of farm management with AMR. Case-control
study: For isolation of indicator *Escherichia (E.) coli*, rectal swab
samples were taken. Cows were sampled depending on treatment history and proximity to one another (cow A: recently treated
parenterally; cow B: untreated, next to cow A; cow C: untreated, at
considerable distance from all treated cows). Antimicrobial susceptibility was
tested by microdilution. Associations of AMR with exposure to cow A, treatments,
and management were analyzed using generalized mixed-effects logistic models. Susceptibility
data on 571 isolates from 131 dairy farms were obtained. Almost no difference
in proportions of resistant *E. coli* was observed between cows B and C (B:
53.4%; C: 57.2%; P=0.52). Untreated cows had lower odds of carrying
resistant *E. coli* than treated cows (B: OR 0.44, P<0.001; C:
OR 0.54, P=0.007). Non-pansusceptibility of isolates was associated with
antimicrobial treatment (1 treatment: OR 2.11, P=0.001; ≥2: OR 1.76, P=0.043).
Using manure on forage crops was associated with higher
odds of pansusceptibility (OR 2.01, P=0.004). For daily practice, with
regard to the risk of AMR transmission, results of this study do not provide
evidence for the need to separate treated cows from others during treatment in
tie-stalls.

1

2 Materials and equipment:

3 Rectal swabs in transport tubes

4 BROLAC agar (Biolab, Budapest, Hungary)

5 MacConkey agar (Thermo Fisher Scientific, Basel, Switzerland)

6 Chromogenic coliform agar (Merck, Darmstadt, Germany)

7 Trypticase soy agar with 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ, USA)

8 Columbia agar with 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ, USA)

9 MALDI-TOF MS (Bruker, Bremen, Germany)

10 Microtubes with glycerol solution (Microbank™, Pro-Lab diagnostics, Wirral, UK)

11 Cation-adjusted Mueller-Hinton broth

12 EUVSEC3 Sensititre™ commercial test plates (Thermo Fisher Scientific, Basel, Switzerland)

13 Incubator set at 37 °C

14 Freezers at -20°C and -80°C

15 Personal protective equipment (lab coat, gloves etc.) Procedure:

16 Sample inoculation:

17 Within 24 hours of collection, remove rectal swabs from their transport tubes.

18 Directly spread the swabs onto the selected agar plates (BROLAC, MacConkey, or Chromogenic coliform agar). Note: ensure samples from the same triplet are inoculated onto the same type of agar.

19 Incubate the plates under aerobic conditions at 37 °C for 24 hours.

20 Colony selection and subculture:

21 After incubation, randomly select one single colony per plate.

22 Streak the selected colony onto trypticase soy agar containing 5% sheep blood or Columbia agar containing 5% sheep blood.

23 Incubate the plates at 37 °C for 24 hours.

24 Identification of E. coli:

25 Perform identification and confirmation of E. coli using MALDI-TOF MS.

26 Storage of isolates:

27 Suspend the confirmed E. coli isolates in microtubes with glycerol solution.

28 Store the microtubes at -20°C initially, and then transfer to -80°C for long-term storage.

29 Antimicrobial susceptibility testing (AST):

30 Thaw the frozen isolates and incubate on Columbia blood agar at 37 °C for 24 hours.

31 Determine the minimum inhibitory concentration (MIC) of antimicrobials using cation-adjusted Mueller-Hinton broth and EUVSEC3 Sensititre™ commercial test plates.

32 Classify the isolates as resistant (R) or susceptible (S) according to EUCAST clinical breakpoints. If not available, use CLSI recommendations.

33 Classification of isolates:

34 Classify isolates as 'pansusceptible' if susceptible to all tested drugs.

35 Classify as 'non-pansusceptible' if resistant to at least one tested drug.

36 Define isolates as 'multidrug-resistant' (MDR) if resistant to at least one drug from a minimum of three different antimicrobial classes. References:

37 EUCAST clinical breakpoints for Enterobacteriales. Available from: https://www.eucast.org/clinical_breakpoints/ CLSI guidelines for antimicrobial susceptibility testing.]. Available from: http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI+M100+ED32%3A2022&sbssok=CLSI+M100+ED32%3A2022+TABLE+2A&format=HTML#CLSI%20M100%20ED32:2022%20TABLE%202A