Characterisation of *Escherichia coli* isolated from Antarctic animals

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Keywords: *Escherichia coli*, classification, typing, Antarctica, animal

INTRODUCTION

Escherichia coli is one of the most commonly used model organisms in various fields of biology. Thanks to its variability and adaptability we can find it all over the world mainly as a part of human and animal microbiome. In the recent years researchers started to explore polar regions under the microscope, including its animal inhabitants, such as seals, sea lions, penguins, skuas, seagulls, sea elephants and cormorants or rather, their microbiome (Power et al. 2016). In this study we used various methods ranging from phenotyping, antibiotic susceptibility testing and molecular biology techniques such as rep-PCR and multiplex-PCR for classification of *E. coli* isolates.

MATERIAL AND METHODS

Bacterial *E. coli* cultures (n = 188) used for this study are property of the Czech Collection of Microorganisms (CCM) and were collected from rectal and cloaca swabs of animals living on Antarctica and Patagonia in the years of 2013-2017. After the primary assessment by the employees of the CCM, we performed the ENTEROtest 24 kitand various additional biochemical tests to supplement the kit results.

Fig. 1. Collection of samples – penguin (*Source*: personal archive of I. Sedláček).

Then, we studied antibiotic susceptibility of *E. coli* strains and also used molecular biology methods such as rep-PCR with primer REP and ERIC and multiplex-PCR methods to assess some virulence factor genes prevalence for: *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *ehly* (enterohemolysin), *eaeA* (intimin), *lt* (thermolabile enterotoxin), STa (thermostable enterotoxin a) and STb (thermostable enterotoxin b). Finally, the Clermont phylogenetic group distribution (Clermont et al. 2000) among analysed strains were achieved.

RESULTS

In the primary assessment, we found out, that some of the *E. coli* strains have a different colony morphology on trypton soya agar (TSA) (Fig. 1 and Fig. 2).

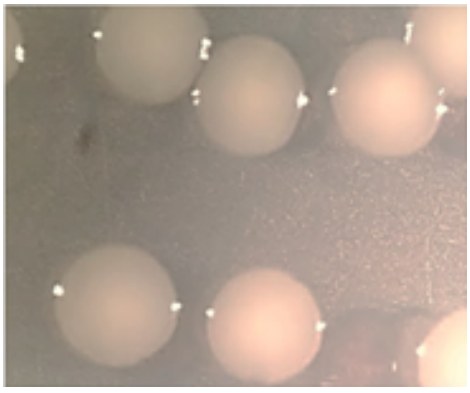

Fig. 2. Common *E. coli* morphology (*Source*: personal archive of G. Suková).

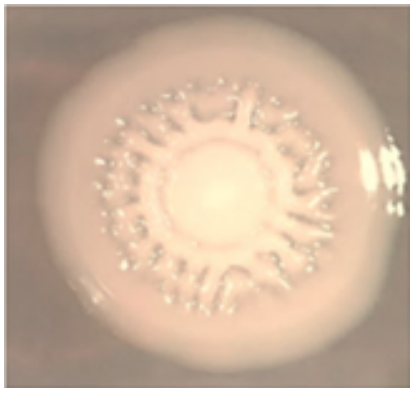

Fig. 3. Uncommon *E. coli* morphology (*Source*: personal archive of G. Suková).

No inordinary results were obtained by assessing the biochemical properties of our samples, but five strains of *E. coli* were found to be multiresistant and n = 71 (37.8 %) strains were intermediary resistant to cephalotin. When using the rep-PCR, we found that the ERIC primer was not suitable for our set of samples, whereas the REP primer worked well and separated strains into individual clusters. In the Clermont group distribution we found the group B2 to be the most common among our samples followed by the group D, B1 and A. Among the virulence factor genes, the most common were STa and STb followed by *stx1*, *eaeA*, LT, *ehly* and *stx2*.

DISCUSSION

Regarding the colony morphology, we did not connect it to any other studied attribute except for the fact these specimen (72.2% of them) were not producing satisfying fingerprints when ERIC-PCR was used. Using the REP-PCR method, we were able to mark some specimen, that could be the same strain isolated twice regarding their fingerprint similarity. Confirming this would require more accurate methods though. REP-PCR dendrogram grouping correlated with Clermont grouping, probably due to the similarities between the strains belonging to same groups. Most surprising was the high

rate of virulence factor genes detected, which did not correlate with Clermont distribution of virulence rates among the individual groups. That could be caused by the fact that Clermont tested strains isolated from humans and our specimen are from animals. The animals could be reservoirs of high viruelnce bacteria either symptomatic or asymptomatic.

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