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Title: Antimicrobial susceptibility testing of historical and recent clinical isolates of *Bordetella pertussis* in the United Kingdom using the Etest method

Article Type: Brief Report

Keywords: *Bordetella pertussis*; antimicrobial resistance; antimicrobial susceptibility; Etest

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Abstract: Reports of the development of antimicrobial resistance by *Bordetella pertussis* to macrolides in the United States and Taiwan, together with a recent increase in pertussis notifications and laboratory confirmed cases in England and Wales in 2008, prompted the examination of historical and recent clinical isolates from patients for evidence of such resistance in our collection. Isolates submitted to our laboratory as part of the enhanced surveillance scheme for pertussis, from 2001 to 2009, were tested against three agents, erythromycin, clarithromycin and azithromycin, by the Etest (bioMérieux) method. All isolates (n = 583) were fully susceptible to all three agents tested (Minimum Inhibitory Concentrations, ≤ 0.125 $\mu\text{g/ml}$). All but one strain (582/583) had MICs of ≤ 0.064 for all three agents. The control strain of *B. pertussis* A228 (from Centers for Disease Control and Prevention, USA) with a resistant phenotype had an MIC of >256 $\mu\text{g/ml}$. Although no evidence of resistance was found in the strains tested from the United Kingdom, screening for antimicrobial resistance of *B. pertussis* may be warranted in cases that are unresponsive to macrolide treatment and to provide early warning of such emergence in the future.

Response to Reviewer 1

This approach, which includes isolates with different serotypes, from different age groups, from geographically diverse locations within UK, and distinct genotypic types would be adequate for surveillance purposes, but taking into account that occurrence of *B. pertussis* resistance to macrolides is estimated to be low, the recommendation would be that a given *B. pertussis* isolate should be tested for resistance only if there is therapeutic failure.

Authors response: This is indeed what we have stated 'sensitivity testing for *B. pertussis* may be warranted in cases that are unresponsive to macrolide treatment '

But we believe that the additional clause 'and to provide early warning of for emergence of such resistance of in the future.' Is fully warranted.

In addition we would argue that evidence showing that *B. pertussis* isolates are sensitive to macrolides is still noteworthy in order to prevent the over-reporting of the occurrence of resistance. Certainly these data have not been previously reported for the UK, however, to accommodate this reviewer's view we have re-formatted the manuscript to a brief report, but would argue that a medical microbiology journal is the most appropriate.

Response to Reviewer 2

This study should be published. It confirms that macrolides are still antibiotics of choice for pertussis infection. The methodology is correct; data are clear and well presented.

Authors response: We thank this reviewer for these positive comments.

Why are the authors using glycerol? The consensus technique was published by Mooi et al., *Eur. J. Clin. Microbiol. Infect. Dis.* 2000. The chromosome of *B. pertussis* being dynamic and containing many IS, glycerol might induce rearrangements.

Authors response: The use of glycerol as a cryoprotectant for preservation of suspensions of microorganisms at low temperature is well-established and is widely used in both commercial and in-house preparations. Our cultures are held at -80°C so these are effectively inert. Strains tested for genotypic markers such as pertactin, pertussis toxin and MLVA type before and after storage show no difference. The mechanism for possible induction of rearrangements of the *B. pertussis* genome due to glycerol is not clear and in the absence of evidence to this effect we cannot comment further. We note that the method suggested by the reviewer uses saccharose, but we are not aware of any data comparing different preservation media on the pertussis genome and any effects of genome rearrangement.

1 BRIEF REPORT

2 **Antimicrobial susceptibility testing of historical and recent clinical**
3 **isolates of *Bordetella pertussis* in the United Kingdom using the**
4 **Etest method**

5 N. K. Fry · J. Duncan · L. Vaghji · R. C. George · T. G. Harrison

6 **Abstract** Reports of the development of antimicrobial resistance by *Bordetella pertussis* to
7 macrolides in the United States and Taiwan, together with a recent increase in pertussis
8 notifications and laboratory confirmed cases in England and Wales in 2008, prompted the
9 examination of historical and recent clinical isolates from patients for evidence of such
10 resistance in our collection. Isolates submitted to our laboratory as part of the enhanced
11 surveillance scheme for pertussis, from 2001 to 2009, were tested against three agents,
12 erythromycin, clarithromycin and azithromycin, by the Etest (bioMérieux) method. All
13 isolates ($n = 583$) were fully susceptible to all three agents tested (Minimum Inhibitory
14 Concentrations, $\leq 0.125 \mu\text{g/ml}$). All but one strain (582/583) had MICs of ≤ 0.064 for all three
15 agents. The control strain of *B. pertussis* A228 (from Centers for Disease Control and
16 Prevention, USA) with a resistant phenotype had an MIC of $>256 \mu\text{g/ml}$. Although no
17 evidence of resistance was found in the strains tested from the United Kingdom, screening
18 for antimicrobial resistance of *B. pertussis* may be warranted in cases that are unresponsive
19 to macrolide treatment and to provide early warning of such emergence in the future.

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21 Key words: *Bordetella pertussis*, antimicrobial resistance, antimicrobial susceptibility, Etest22
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25 Infections, 61 Colindale Avenue, London NW9 5EQ, United Kingdom26 e-mail: Norman.Fry@HPA.org.uk

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1 Susceptibility testing of *Bordetella* spp. including *B. pertussis* clinical isolates is not routinely
2 performed and prior to 1994 resistance to erythromycin in *B. pertussis* had not been
3 observed [1]. However, since then there have been a few reports presenting the emergence
4 of erythromycin-resistant *B. pertussis* isolates in the USA [2,3] and one in Taiwan [4].
5 These reports and a recent increase in notifications [5], prompted this examination of our
6 collection of historical and recent clinical isolates of *B. pertussis*. Clinical isolates of *B.*
7 *pertussis* are submitted to our laboratory, the Respiratory and Systemic Infection Laboratory
8 (RSIL) as part of the enhanced surveillance scheme for pertussis for England and Wales. Our
9 laboratory also receives isolates from Ireland, Northern Ireland and Scotland, and these
10 were also included in this analysis. In this study, we report the susceptibility testing to
11 erythromycin, clarithromycin and azithromycin by the Etest (bioMérieux) method for 583 *B.*
12 *pertussis* clinical isolates that represent the majority (≥ 95 %) of isolates submitted to the
13 RSIL between January 2004 and December 2009, and a selected number of isolates from
14 September 2001 to December 2003. The years of isolation and numbers of strains isolated
15 were as follows: 2001, 2; 2002, 40; 2003, 17; 2004, 68; 2005, 110; 2006, 53; 2007, 69; 2008,
16 134; and 2009, 90. Prior to testing isolates were initially subcultured into Charcoal blood
17 agar (Media Services, Central Public Health Laboratory, London) to check for purity and
18 incubated at 37°C for 24-72 hrs. Mueller Hinton agar plates supplemented with 10% horse
19 blood (Oxoid, CPHL Media Services), 25 ml final volume, 90 mm triple-vented Petri dish
20 (Greiner Bio-One Ltd), were inoculated by resuspension of bacterial growth as
21 recommended for *Bordetella* spp. testing, but modified to use a higher MacFarland
22 equivalent of 8, to achieve the semi-confluent growth required. E-strips (bioMérieux) were
23 equilibrated to room temperature then carefully applied to the centre of each agar plate.
24 Inoculated plates were incubated for 48 h to 7 days in a moist atmosphere at 37°C. The
25 minimum inhibitory concentrations (MICs) were determined by using the Etest
26 methodology. The following compounds were tested erythromycin (Cat. No. 1058),
27 clarithromycin (Cat. No. 0878), and azithromycin (Cat. No. 0168). Control strains of
28 erythromycin resistant *B. pertussis* A228 (a gift from Garry Sanden, Centers for Disease
29 Control and Prevention, Atlanta, Georgia, USA) and the Oxford *Staphylococcus aureus*
30 NCTC6571 (National Collection of Type Cultures, London) were included in each batch.

1 The MIC values of test and control strains of *B. pertussis* were recorded at 72 h, following
2 the manufacturer's instructions. In addition the plates were examined at 7 days to check for
3 the appearance of the heterogenous phenotype for erythromycin resistance [6]. Isolates of
4 *B. pertussis* with MICs of >256 µg/ml after 72 h were considered be resistant [2].

5 The *B. pertussis* isolates tested (from 2001-2009) included different serotypes (11 serotype
6 1,2; 569 serotype 1,3; and 11 untypable) from different age groups (<3 month, 348; 3-5
7 months, 90; 6-11 months, 23; 1-4 years, 1; 5-9 years, 5; 10-14 years, 10; ≥15 years, 15;
8 unknown, 24) with a male:female ratio of 1:1.1. Isolates were received from geographically
9 diverse locations within the United Kingdom and were known to include a number of
10 distinct genotypic types, i.e., pertactin, pertussis toxin, Multi-locus Variable Number of
11 Tandem Repeats Analysis [7], and authors' unpublished data. All isolates (523/523) were
12 susceptible to all three agents tested (MICs ≤0.125 µg/ml) with the Etest method. An
13 example of the results obtained is shown in the Figure. All isolates had MICs of ≤0.064 µg/ml
14 with both erythromycin and azithromycin and one strain had an MIC of 0.125 µg/ml with
15 clarithromycin. These results are comparable to those obtained with these agents by a study
16 of 36 strains from Northern California by the Etest method [8]. No evidence of
17 heterogeneous resistance was found. The erythromycin resistant *B. pertussis* A228 strain
18 always yielded an MIC value of >256 µg/ml with all three agents, which is consistent with
19 that previously reported [2]. The control strain NCTC6571 gave results consistent with target
20 MICs for all three of these agents i.e., 0.12 µg/ml [9].

21 Macrolides (especially erythromycin) remain the current antibiotics of choice for post-
22 exposure prophylaxis and treatment of pertussis infection [10]. Azithromycin is also
23 recommended for prophylaxis of neonates as it is associated with fewer adverse effects
24 [11]. The data from this study confirm that the current guidelines for macrolide treatment
25 remain appropriate for *B. pertusiss* [11-13]. There are few documented occurrences of *B.*
26 *pertussis* resistance to macrolides to date and the frequency of occurrence is estimated to
27 be low (<1%) [6]. Although no evidence of macrolide resistance was found in this study of a
28 large ($n = 583$) number of clinical isolates from the UK, sensitivity testing for *B. pertussis* may
29 be warranted in cases that are unresponsive to macrolide treatment and to provide early
30 warning of for emergence of such resistance of in the future. The mechanism of resistance

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1 was proposed to be due to a mutation of the erythromycin binding site in the 23S rRNA
2 gene, and identification of this and other potential resistance mechanism should allow
3 genotypic testing and facilitate more rapid screening of large numbers of isolates or clinical
4 specimens in the future.

5

6 **Acknowledgments** We thank Dr Garry Sanden for his kind gift of the erythromycin resistant
7 *B. pertussis* A228 strain, Zak Prior for assistance with the Figure, and staff from all
8 laboratories who have submitted *B. pertussis* isolates.

9 **Conflicts of interest statement**

10 None

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1 References

- 2 1. Lewis K, Saubolle MA, Tenover FC, Rudinsky MF, Barbour SD, Cherry JD (1995)
3 Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. *Pediatr*
4 *Infect Dis J* 14:388-391
- 5 2. Bartkus JM, Juni BA, Ehresmann K, Miller CA, Sanden GN, Cassiday PK, Saubolle M,
6 Lee B, Long J, Harrison AR, Besser JM (2003) Identification of a mutation associated
7 with erythromycin resistance in *Bordetella pertussis*: implications for surveillance of
8 antimicrobial resistance. *J Clin Microbiol* 41:1167-1172
- 9 3. Korgenski EK, Daly JA (1997) Surveillance and detection of erythromycin resistance in
10 *Bordetella pertussis* isolates recovered from a pediatric population in the
11 intermountain west region of the United States. *J Clin Microbiol* 35:2989-2991
- 12 4. Yao S-M, Liaw G-J, Chen Y-Y, Yen M-H, Chen Y-H, Mu J-J, Chiang CS (2008)
13 Antimicrobial susceptibility testing of *Bordetella pertussis* in Taiwan prompted by a
14 case of pertussis in a paediatric patient. *J Med Microbiol* 57:1577-1580
- 15 5. HPA (2009). Statutory Notifications of Infectious Diseases (NOIDs) – Annual Totals
16 1994 to 2008 - England and Wales. Health Protection Agency, London.
17 http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1251473364307. Accessed 1
18 April 2010
- 19 6. Wilson KE, Cassiday PK, Popovic T, Sanden GN (2002) *Bordetella pertussis* isolates
20 with a heterogeneous phenotype for erythromycin resistance. *J Clin Microbiol*
21 40:2942-2944
- 22 7. Litt DJ, Neal SE, Fry, NK (2009) Changes in genetic diversity of the UK *Bordetella*
23 *pertussis* population between 1920 and 2006 reflect vaccination coverage and the
24 emergence of a single dominant clonal type. *J Clin Microbiol* 47:680-688
- 25 8. Gordon KA, Fusco J, Biedenbach DJ, Pfaller MA, Jones RN (2001) Antimicrobial
26 susceptibility testing of clinical isolates of *Bordetella pertussis* from northern
27 California: report from the SENTRY antimicrobial surveillance program. *Antimicrob*
28 *Agents Chemother* 45:3599-3600
- 29 9. Andrews JM (2010) BSAC Methods for Antimicrobial Susceptibility Testing.
30 http://www.bsac.org.uk/_db/_documents/Version_9.1_March_2010_final.pdf.
31 Accessed 1 April 2009
- 32 10. Murphy TV, Slade BA, Broder KR, Kretsinger K, Tiwari T, Joyce PM, Iskander JK, Brown
33 K, Moran JS (2008) Prevention of pertussis, tetanus, and diphtheria among pregnant
34 and postpartum women and their infants: recommendations of the Advisory
35 Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 57:1-47, 51.
36 <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5704a1.htm>. Accessed 1 April
37 2010

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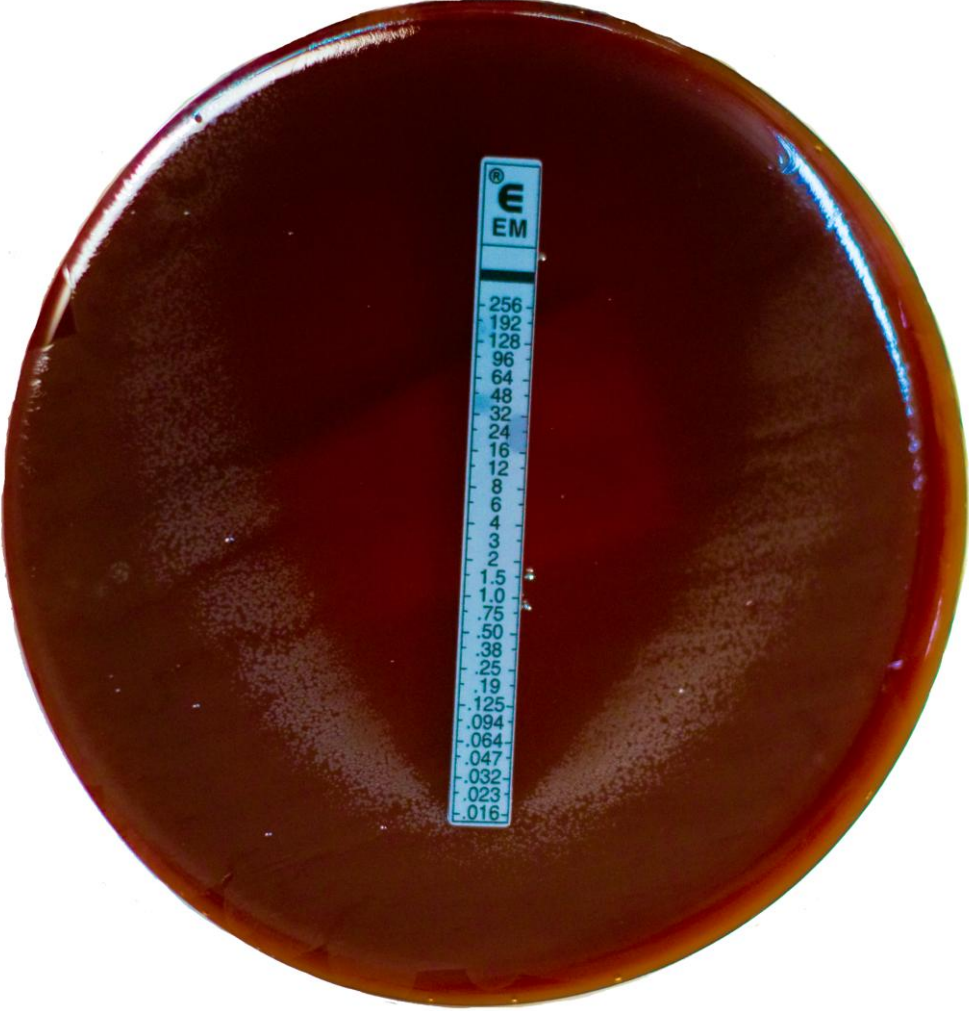
11. Tiwari T, Murphy TV, Moran J (2005) Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. MMWR Recomm Rep 54:1-16

12. Gonzales R, Bartlett JG, Besser RE, Cooper RJ, Hickner JM, Hoffman JR, Sande MA (2001) Principles of appropriate antibiotic use for treatment of uncomplicated acute bronchitis: background. Ann Intern Med 134:521-529

13. Dodhia H, Crowcroft NS, Bramley JC, Miller E (2002) UK guidelines for use of erythromycin chemoprophylaxis in persons exposed to pertussis. J Public Health Med 24:200-206

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- 1 **Figure.** Example of results from a UK clinical *B. pertussis* strain subcultured on Mueller
- 2 Hinton agar plate supplemented with 10% horse blood challenged with the erythromycin
- 3 Etest strip after 72 h incubation, showing zone of clearance.



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