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Clavulanic acid can be the component in amoxicillin-clavulanic acid responsible for immediate hypersensitivity reactions

To the Editor:

Immediate allergic reactions to penicillins usually appear within 1 hour after drug intake and are mediated by specific IgE antibodies.¹ Amoxicillin is the penicillin most frequently involved in sensitization, with the side chain playing a relevant role.¹ Amoxicillin is commercialized alone or combined to clavulanic acid (CLV). Although initial studies showed that CLV had a low immunogenic capacity,² allergic reactions to this compound may exist.^{3,4}

We evaluated all patients (N = 307) seen at our allergy department for an allergic reaction after administration of amoxicillin-clavulanic acid (AX-CLV) over 3 years (2006-2008). Those finally diagnosed as having had an immediate allergic reaction and with a positive skin test were included in this study. Thirty cases with good tolerance to AX-CLV were used as controls. The study was approved by the institutional review board, and informed consent was obtained.

Skin testing was performed as described⁵ by using benzylpenicilloyl-polylysine (PPL; 5×10^{-5} M), minor determinant mixture (2×10^{-2} M), and CLV (20 mg/mL), all provided by Diater (Madrid, Spain), and amoxicillin (GlaxoSmithKline Beecham, Madrid, Spain; 20 mg/mL). In those cases with a positive skin test to CLV, AX-CLV (GlaxoSmithKline Beecham) was also tested (20 mg/mL amoxicillin and 4 mg/mL CLV).

Single-blind, placebo-controlled drug provocation testing (DPT) was done by using benzylpenicillin, amoxicillin, and AX-CLV, as reported.⁵ This approach enabled us to classify patients as allergic to benzylpenicillin, amoxicillin, or CLV.

Basophil activation testing (BAT) was done as described,⁶ with benzylpenicillin, amoxicillin, AX-CLV, and CLV at 4 different concentrations (this article's Fig E1 in the Online Repository at www.jacionline.org shows the dose-response curve). Results were considered positive when the stimulation index (SI), calculated as a ratio between the percentage of activated basophils with the different haptens and the negative control, was ≥ 2 to at least one of the concentrations mentioned.

To confirm that the drug-induced basophil activation was IgE-mediated, we analyzed the wortmannin inhibitory effect at different concentrations (5, 1, 0.1 $\mu\text{mol/L}$) with benzylpenicillin, amoxicillin, AX-CLV, CLV, and the positive controls (chemotactic peptide N-Formyl-Met-Leu-Phe [fMLP] and anti-IgE).⁷ We also followed up the BAT-positive patients at 6-month intervals over 1 year to evaluate the specific IgE clearance.

Comparisons for quantitative variables without a normal distribution were done by the Kruskal-Wallis tests using SPSS (15.0; IBM, Chicago, Ill).

Of the initial group of 307 patients evaluated with a reaction attributed to AX-CLV, 31 refused or did not complete the study.

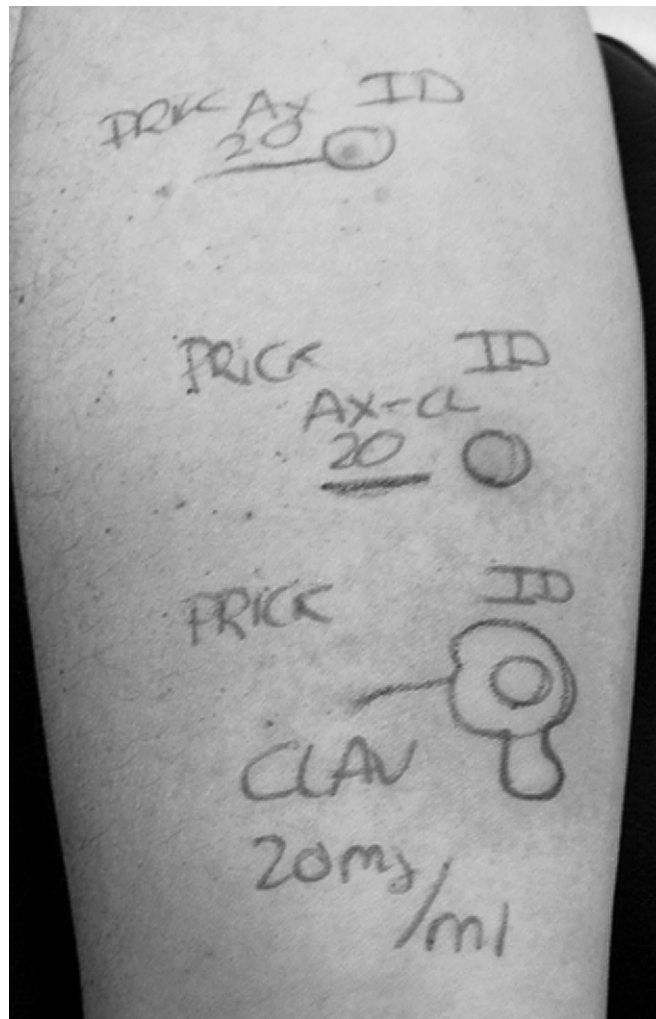


FIG 1. Skin test results using amoxicillin (20 mg/mL), amoxicillin-clavulanic acid (20/4 mg/mL), and clavulanic acid (20 mg/mL) in patient 55C. On the *left* are shown prick results (all negative) and on the *right* intradermal results (only positive to clavulanic acid).

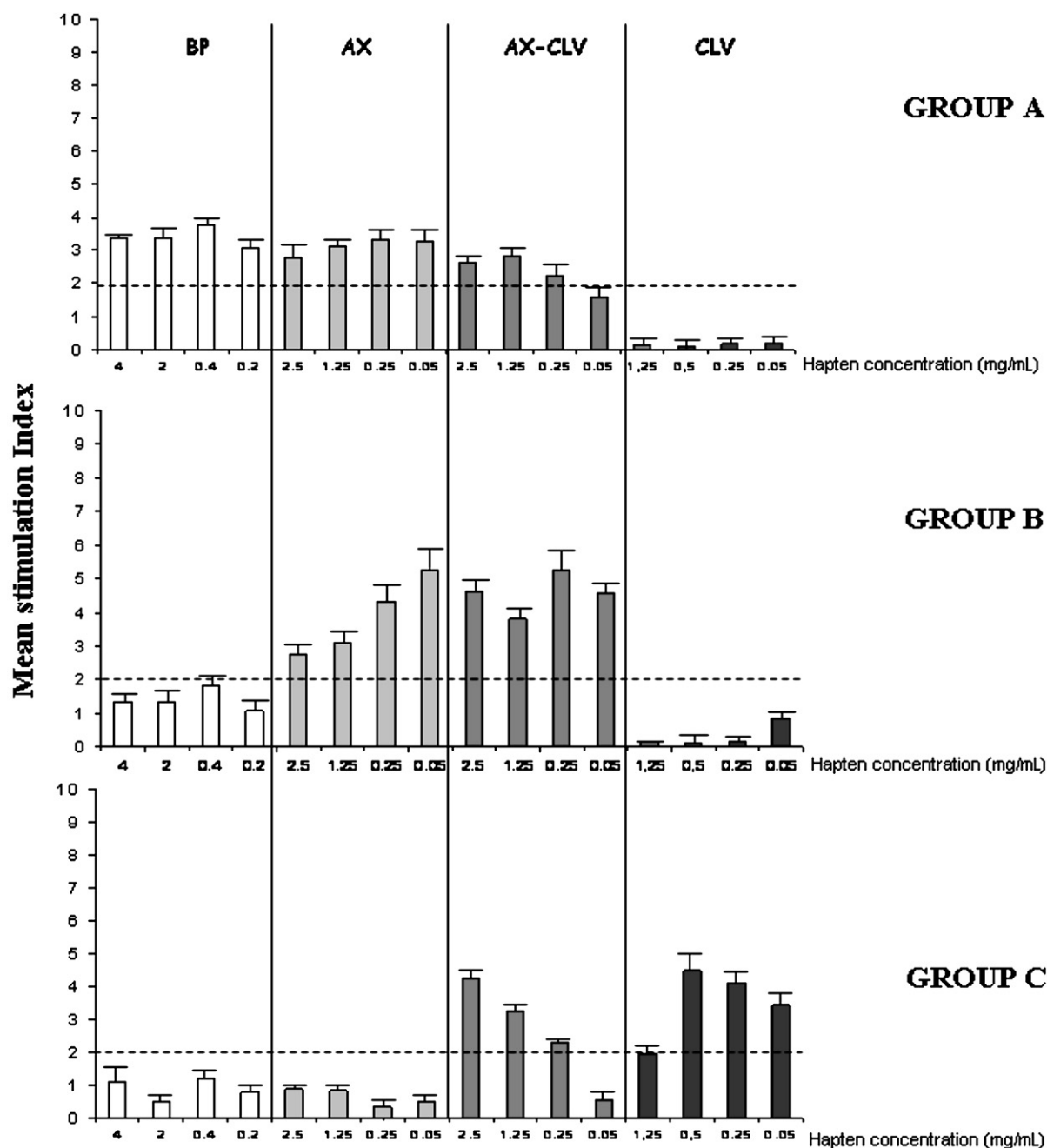


FIG 2. Mean and SD of SI from BAT in positive cases in the 3 groups, with benzylpenicillin (BP), amoxicillin (AX), AX-CLV, and CLV at different concentrations (mg/mL). The AX-CLV concentration expresses the AX concentration in the combination.

Of the remaining 276, fifty-five (19.9%) had a positive skin test result to different penicillin determinants. Those with negative skin tests (N = 221) underwent DPT, with the following results: 199 had good tolerance to benzylpenicillin, amoxicillin, and AX-CLV and were considered not to have allergy; 15 had good tolerance to benzylpenicillin, developed an immediate reaction to amoxicillin, and were considered allergic to amoxicillin; and 7 had good tolerance to benzylpenicillin and amoxicillin, developed an immediate reaction to AX-CLV, and were considered allergic to CLV.

Those with positive skin tests (N = 55) were subjected to further analysis and classified in 3 groups, according to skin test results and DPT: group A (N = 5; 9%), patients with a positive skin test result to PPL or minor determinant mixture; group B (N = 34; 62%), patients with a negative skin test result to PPL and minor determinant mixture, good tolerance to benzylpenicillin by DPT, and a positive skin test to amoxicillin; and group C (N = 16; 29%), patients with a negative skin test result to PPL, minor determinant mixture and amoxicillin, good tolerance by DPT to benzylpenicillin and amoxicillin, and a positive skin test to CLV.

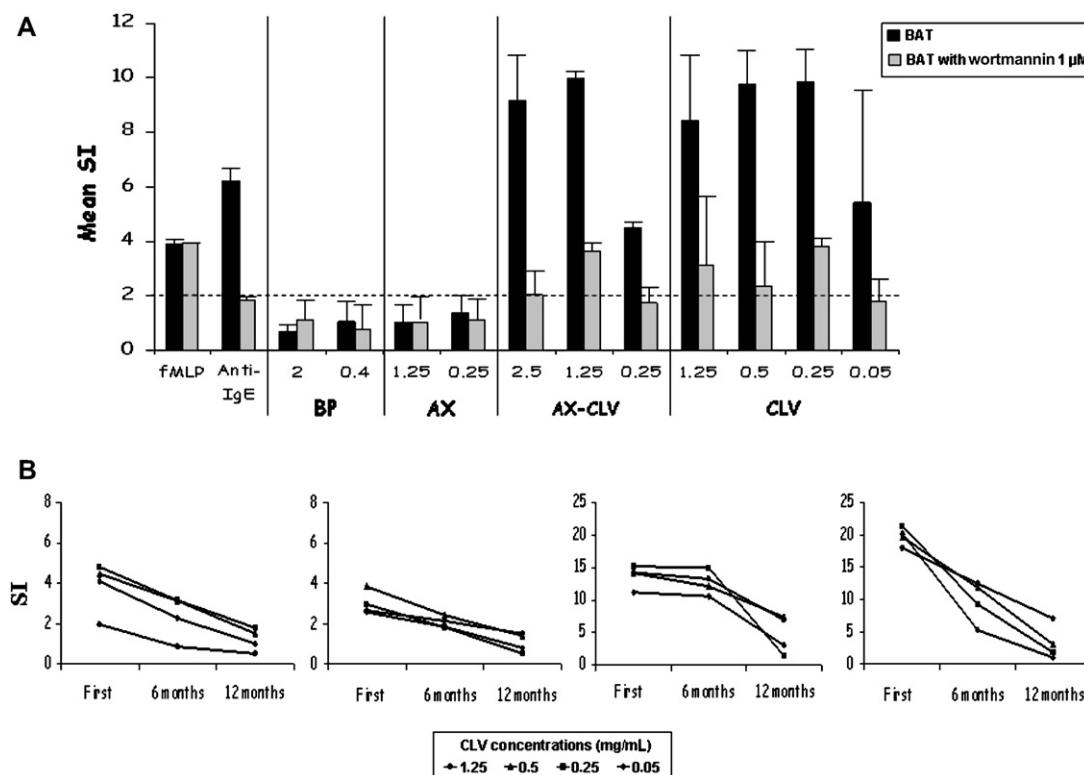


FIG 3. A, Wortmannin inhibitory effect in basophil activation under different conditions: positive controls (fMLP and anti-IgE), benzylpenicillin (BP), amoxicillin (AX), AX-CLV, and CLV at different concentrations (mg/mL). These data show the mean SI from 4 patients of group C. **B,** BAT results at different CLV concentrations (1.25, 0.5, 0.25, and 0.05 mg/mL) during a follow-up period of 12 months. These figures represent individual data from 4 patients of group C.

In group C, skin testing with AX-CLV was also performed and was positive in 10 cases (Fig 1; see this article's Table E1 in the Online Repository at www.jacionline.org). Comparisons between groups showed significant differences for age ($P < .005$), with group A the oldest and group C the youngest.

The BAT was done in all 55 patients and was positive in 29 (52.7%): 3 (60%) in group A, 18 (52.9%) in group B, and 8 (50%) in group C. The mean SI values in the positive cases from each group are shown in Fig 2. The BAT was also done in the control group, showing 90% specificity. We observed that the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, induced BAT inhibition when stimulated with anti-IgE and with haptens, especially in those patients with positive BAT results, but not when fMLP was used as the basophil stimulator (Fig 3, A). Moreover, the BAT decreased, even becoming negative, in those cases with low BAT positivity (Fig 3, B).

Although CLV has a β -lactam ring able to bind covalently to proteins, it has important chemical differences with penicillin antibiotics: it lacks the side chain, and the thiazolidine ring has been substituted by an oxazolidine. These differences may contribute to the generation of allergenic determinants with little or no cross-reactivity with those generated by benzylpenicillin or amoxicillin. With the increased consumption of AX-CLV, more and more patients have appeared with immediate reactions to this combination, with most studies indicating that amoxicillin was the inducer.^{1,8} Evidence about whether CLV could be the culprit drug is so far limited to just a few case reports.^{3,4}

In our study, 20% of the patients were confirmed as having had an immediate allergic reaction to AX-CLV with a positive skin test to 1 or more of the reagents used. We found that just 9% (group A) recognized benzylpenicillin determinants, and an important percentage (62%) recognized amoxicillin determinants. This indicates that amoxicillin is still the most frequently involved penicillin in inducing sensitization.^{1,8} Notably, whereas 30% of patients had a positive skin test result to CLV and had good tolerance to amoxicillin, only 18% were detected by AX-CLV. Because skin test sensitivity to β -lactam determinants is concentration-dependent, we believe these differences are related to the CLV concentration used (20 mg/mL when used alone vs 4 mg/mL when AX-CLV was used). Because AX-CLV has so far been the only drug available for skin testing, this may explain why more reactions with a positive skin test result to CLV have not been reported.

The pattern of responses observed in the BAT studies confirmed the existence of these 3 groups, with patients recognizing penicillin determinants, patients recognizing amoxicillin-specific determinants, and patients specifically recognizing CLV determinants. Because basophil activation could be induced by non-IgE-mediated mechanisms, we tried to confirm the involvement of specific IgE in our cases by using a PI3K inhibitor, wortmannin, under different basophil stimulation conditions. PI3K is one of the important kinases activated by Fc ϵ RI receptor cross-linking and is involved in regulating histamine release.⁹ Although PI3K inhibition with wortmannin can also inhibit IL-3 and

GM-CSF-induced activation of basophils, these cytokines do not induce activation, nor do they induce CD63. In addition, it is unlikely that IL-3, GM-CSF, or even monocyte chemoattractant protein MCP 1 activities were present in our drug solutions. Therefore, we consider that our results with wortmannin and the negativization over time support an IgE-mediated response.

Our results indicate that immediate selective reactions to CLV do exist and account for around 30% of immediate allergic reactions in cases that are positive after taking the combination of AX-CLV. We recommend using CLV in the diagnostic evaluation in cases of immediate reactions in which the association AX-CLV is incriminated when conventional skin testing with benzylpenicillin and amoxicillin determinants proves negative.

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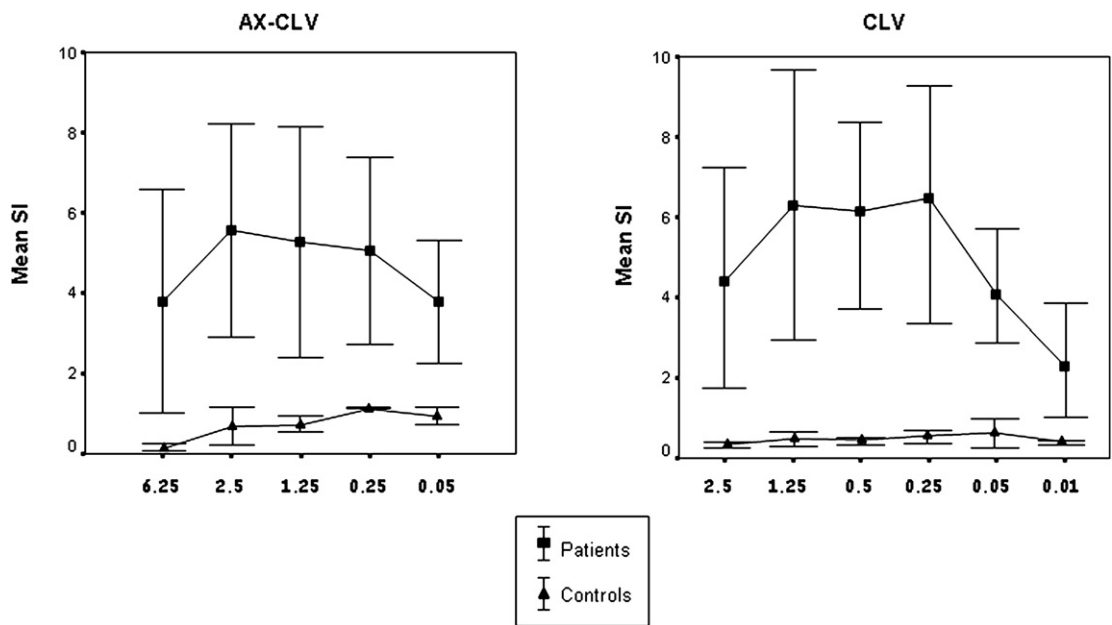


FIG E1. Dose -response curve for positive patients (N = 8) and controls (N = 8), responders to AX-CLV (A) or CLV (B). Results are expressed as the SIs (means ± SEMs).

TABLE E1. Clinical characteristics and skin test and drug provocation test results of the study patients

Patient no.	Age (y)	Sex	Interval (mo)	Reaction	Skin test (wheal increase in mm)					DPT	
					PPL	MDM	AX	CLV	AX-CLV	BP	AX
1A	61	F	6	Urticaria	ID (+) (3 × 3)	(-)	(-)	(-)	ND	ND	ND
2A	47	F	5	Urticaria	(-)	ID (+) (3 × 4)	ID (+) (5 × 6)	(-)	ND	ND	ND
3A	63	M	1	Anaphylaxis	(-)	P (+) (4 × 3)	P (+) (4 × 4)	(-)	ND	ND	ND
4A	59	F	1	Anaphylaxis	ID (+) (5 × 7)	ID (+) (4 × 6)	P (+) (5 × 6)	(-)	ND	ND	ND
5A	48	M	18	Anaphylaxis	(-)	P (+) (4 × 5)	P (+) (4 × 3)	(-)	ND	ND	ND
6B	49	F	4	Anaphylaxis	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
7B	20	F	2	Urticaria	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
8B	37	M	9	Urticaria	(-)	(-)	ID (+) (3 × 3)	(-)	ND	(-)	ND
9B	55	F	5	Anaphylaxis	(-)	(-)	ID (+) (4 × 6)	(-)	ND	(-)	ND
10B	41	F	7	Urticaria	(-)	(-)	ID (+) (3 × 3)	(-)	ND	(-)	ND
11B	42	M	4	Anaphylaxis	(-)	(-)	ID (+) (5 × 7)	(-)	ND	(-)	ND
12B	52	F	6	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
13B	47	F	5	Anaphylaxis	(-)	(-)	P (+) (4 × 5)	(-)	ND	(-)	ND
14B	30	M	24	Anaphylaxis	(-)	(-)	ID (+) (5 × 7)	(-)	ND	(-)	ND
15B	46	M	46	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
16B	19	F	12	Anaphylaxis	(-)	(-)	ID (+) (4 × 6)	(-)	ND	(-)	ND
17B	57	M	2	Anaphylaxis	(-)	(-)	P (+) (4 × 5)	(-)	ND	(-)	ND
18B	45	F	4	Urticaria	(-)	(-)	ID (+) (3 × 3)	(-)	ND	(-)	ND
19B	46	M	6	Anaphylaxis	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
20B	31	M	5	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
21B	37	M	4	Anaphylaxis	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
22B	54	M	2	Anaphylaxis	(-)	(-)	ID (+) (5 × 5)	(-)	ND	(-)	ND
23B	38	F	1	Anaphylaxis	(-)	(-)	P (+) (4 × 5)	(-)	ND	(-)	ND
24B	67	M	2	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
25B	46	M	1	Anaphylaxis	(-)	(-)	P (+) (4 × 6)	(-)	ND	(-)	ND
26B	60	F	1	Anaphylaxis	(-)	(-)	P (+) (4 × 5)	(-)	ND	(-)	ND
27B	60	M	1	Anaphylaxis	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
28B	18	M	2	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
29B	47	F	4	Anaphylaxis	(-)	(-)	P (+) (4 × 4)	(-)	ND	(-)	ND
30B	53	M	2	Urticaria	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
31B	36	F	5	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
32B	41	M	4	Anaphylaxis	(-)	(-)	ID (+) (5 × 6)	(-)	ND	(-)	ND
33B	35	M	6	Anaphylaxis	(-)	(-)	ID (+) (4 × 5)	(-)	ND	(-)	ND
34B	44	F	12	Anaphylaxis	(-)	(-)	ID (+) (3 × 4)	(-)	ND	(-)	ND
35B	47	M	1	Anaphylaxis	(-)	(-)	P (+) (4 × 5)	(-)	ND	(-)	ND
36B	59	M	1	Anaphylaxis	(-)	(-)	ID (+) (5 × 5)	(-)	ND	(-)	ND
37B	55	M	1	Anaphylaxis	(-)	(-)	P (+) (3 × 4)	(-)	ND	(-)	ND
38B	48	M	2	Anaphylaxis	(-)	(-)	P (+) (4 × 4)	(-)	ND	(-)	ND
39B	57	M	1	Anaphylaxis	(-)	(-)	ID (+) (3 × 3)	(-)	ND	(-)	ND
40C	42	F	22	Anaphylaxis	(-)	(-)	(-)	ID (+) (3 × 3)	ID (+) (3 × 4)	(-)	(-)
41C	26	M	5	Anaphylaxis	(-)	(-)	(-)	ID (+) (3 × 5)	ID (+) (3 × 4)	(-)	(-)
42C	59	F	4	Anaphylaxis	(-)	(-)	(-)	ID (+) (5 × 5)	ID (+) (3 × 4)	(-)	(-)
43C	18	M	2	Urticaria	(-)	(-)	(-)	P (+) (5 × 6)	ID (+) (5 × 6)	(-)	(-)
44C	35	M	2	Anaphylaxis	(-)	(-)	(-)	ID (+) (4 × 6)	ID (+) (4 × 4)	(-)	(-)
45C	56	F	12	Urticaria	(-)	(-)	(-)	ID (+) (5 × 6)	(-)	(-)	(-)
46C	42	M	1	Anaphylaxis	(-)	(-)	(-)	P (+) (4 × 4)	P (+) (4 × 3)	(-)	(-)
47C	30	M	11	Anaphylaxis	(-)	(-)	(-)	ID (+) (3 × 4)	(-)	(-)	(-)
48C	30	M	3	Anaphylaxis	(-)	(-)	(-)	ID (+) (5 × 4)	ID (+) (3 × 4)	(-)	(-)
49C	18	M	1	Anaphylaxis	(-)	(-)	(-)	ID (+) (4 × 6)	ID (+) (5 × 5)	(-)	(-)
50C	38	F	10	Anaphylaxis	(-)	(-)	(-)	ID (+) (4 × 4)	(-)	(-)	(-)
51C	47	F	4	Urticaria	(-)	(-)	(-)	ID (+) (5 × 5)	(-)	(-)	(-)
52C	49	F	1	Anaphylaxis	(-)	(-)	(-)	P (+) (5 × 4)	P (+) (3 × 4)	(-)	(-)
53C	28	M	7	Anaphylaxis	(-)	(-)	(-)	ID (+) (6 × 4)	ID (+) (3 × 4)	(-)	(-)
54C	34	M	10	Anaphylaxis	(-)	(-)	(-)	ID (+) (5 × 7)	(-)	(-)	(-)
55C	31	F	15	Anaphylaxis	(-)	(-)	(-)	ID (+) (4 × 7)	(-)	(-)	(-)

AX, Amoxicillin; BP, benzylpenicillin; F, female; ID, intradermal; M, male; MDM, minor determinant mixture; ND, not done; P, prick.