

Original article

Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing

Background: Penicillin is no longer the most commonly prescribed β -lactam, and the pattern of reactions has changed. We studied the diagnostic value of skin testing in penicillin-allergic subjects from a population where benzylpenicillin is not now the most frequently used β -lactam.

Methods: Patients with a history of immediate allergic reactions to penicillins were studied with: skin tests with major and minor determinants of benzylpenicillin (BPO/MDM), amoxicillin, and ampicillin; *in vitro* determination of specific IgE; and controlled administration for those with a positive history but negative skin and *in vitro* tests. A reaction was considered immediate when symptoms appeared within a maximum of 1 h after drug intake.

Results: After testing, 290 patients (71% having anaphylaxis and 29% having urticaria) proved to be allergic. Amoxicillin was involved in 64.8% and benzylpenicillin in 2.8% of the patients. Skin test positivity to at least one determinant appeared in 70% of cases, amoxicillin being the most frequent. The overall sensitivity decreased markedly when only BPO and MDM were considered. In 13.1% of patients, the diagnosis was established by *in vitro* test and in 16.9% by controlled administration. Of the 290 patients, 42.1% were positive to determinants generated from benzylpenicillin and 57.9% were selective responders.

Conclusions: Sensitivity of skin tests to BPO was lower than reported, being partly replaced by minor determinants, mostly amoxicillin. The incorporation of additional reagents and the development of new tests are required, and these will probably change as the patterns of consumption vary.

**M. J. Torres¹, A. Romano²,
C. Mayorga¹, M. C. Moya³,
A. E. Guzman¹, M. Reche⁵,
C. Juarez⁴, M. Blanca⁵**

¹Research Unit for Allergic Diseases, Carlos Haya Hospital, Malaga, Spain; ²Allergy Service, Catholic University of Rome, Italy; ³Allergy Unit, Hospital Torrecardenas, Almeria, Spain; ⁴Internal Medicine Department, Carlos Haya Hospital, Malaga, Spain; ⁵Allergy Service, University Hospital La Paz, Madrid, Spain

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Miguel Blanca
Servicio de Alergología
Hospital Universitario "La Paz"
Paseo de La Castellana 261
28046 Madrid
Spain

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Allergic reactions to β -lactams are the most common cause of adverse drug reactions mediated by specific immunologic mechanisms (1). The initial decrease in the rate of reactions to β -lactams has been progressively compensated for by the much wider use of these antibiotics (2, 3). Furthermore, the world of β -lactams has become more dynamic and complex due to the increased number of chemical structures available (4). Although β -lactams have been reported to have a decreased capacity for inducing immunologic reactions, including IgE responses, the increased exposure of the population has resulted in a relevant number of allergic reactions every year (3, 5). These reactions may be produced by all β -lactams currently available, ranging from benzylpenicillin (BP), the classical representative (6, 7), to others more recently introduced such as aztreonam or clavulanic acid (8, 9). Not only may the

immunogenicity of each β -lactam chemical structure be different, but the pattern of consumption of the drugs also varies from country to country, thus influencing the appearance of reactions (10). Various investigators have shown interest in studying allergy to β -lactams, and it is expected that more groups will be involved and that further progress in this field will be made (3–12).

Our group has been working in this area of research since the mid-1980s, and a large database is available in which we have registered and followed an important cohort of patients allergic to β -lactams. Consequently, we are in a position not only to consider clinical and laboratory data but also to analyze tendencies over years. In this study, only those subjects with a confirmed immediate allergic reaction to a penicillin derivative were included. Nonimmediate reactions and reactions to other β -lactams, such as cephalosporins or monobactams, were excluded because these will be studied separately. Our results confirm initial studies by our group and others (4, 12–15), showing that benzylpenicilloyl (BPO) is no longer the most relevant hapten in immediate allergic reactions to penicillins.

Abbreviations. BP: benzylpenicillin; BPO: benzylpenicilloyl; AX: amoxicillin; BPO-PLL: benzylpenicilloyl-poly-L-lysine; AXO-PLL: amoxicilloyl-poly-L-lysine; MDM: minor determinant mixture; AMP: ampicillin.

This indicates that the diagnostic accuracy of skin tests with BPO has decreased, so that the use of other determinants, such as amoxicillin (AX), seems to be necessary.

Material and methods

Patients and controls

All subjects referred to our clinic from family practice or emergency departments as a consequence of having had an immediate allergic reaction to a penicillin derivative, administered by either the oral or parenteral route, over a period of 10 years (1985–95) were eligible for inclusion in the study. Since allergy to penicillin is reported in many circumstances, and many cases are falsely labeled as positive, only those with a clearly immediate allergic reaction confirmed after completing the protocol described below were included. A reaction was considered immediate when symptoms appeared within a maximum of 1 h after drug intake, and these reactions were classified into two categories: urticaria or anaphylaxis. Urticaria was defined as an erythematous area with large wheals at different sites on the body; anaphylaxis was considered to exist when more than one organ was affected (with, for example, generalized erythema, dyspnea, abdominal cramps, hoarseness) with or without documented hypotension immediately after penicillin administration (10).

To establish the criteria for specificity in the skin tests, we used 120 age- and sex-matched subjects as a negative control group. Good tolerance to different penicillins was established in all by skin tests and controlled administration of the drug by the same method as for the patients.

Informed consent and approval by the ethics committee were obtained in all cases and controls.

Specific IgE antibody determination by Pharmacia CAP-FEIA System

This is an *in vitro* test system based on ImmunoCAP technology for determination of circulating specific IgE antibodies. The test was carried out as recommended by the manufacturer (Pharmacia, Uppsala, Sweden). The drugs used were c1 (BPO) and c6 (AXO). The Pharmacia CAP-FEIA System specific IgE measuring range was 0.35–100 kU_A/l, and the cutoff values were ≥ 0.35 kU_A/l for positive test results and < 0.35 kU_A/l for negative test results.

Skin test

The determinants and the final concentrations used for skin tests were as follows: BPO, 5×10^{-5} mmol/l; minor determinant mixture (MDM), composed of BP and benzylpenicilloic acid, 2×10^{-2} mmol/l (both provided by Allergopharma, Merck, Darmstadt, Germany); AX, 20 mg/ml (Beecham, Toledo, Spain); and ampicillin (AMP), 20 mg/ml (Antibiotic SA, León, Spain). Tests were all carried out as previously described (10, 16), with extracts freshly reconstituted and taken directly from the vial. Skin tests were made by prick, and, if responses were negative, intradermal tests were carried out. Responses were classified as positive or negative as previously described (17). In the skin prick tests, a wheal larger than 2 mm accompanied by erythema with a negative response to the control saline was considered positive. In the intradermal tests, the wheal area was marked initially and 20 min after testing, and an increase in diameter greater than 3 mm was considered positive. The inclusion of AX and AMP from the very beginning was based on previous evidence of side-chain-specific reactions to AX (10, 18–20). In patients who reported symptoms compatible with severe reactions, or who had experienced mild symptoms but were at special risk, the

prick test was followed by intradermal 10 000-fold dilutions, which were gradually increased until a positive skin response occurred or until the final concentration described above was reached.

Controlled administration

Single-blind, placebo-controlled administration, performed in those patients with both skin test and RAST negative, was done as previously described (10, 16) with some modifications. BP was administered parenterally at the following doses: 1 ml at 10^3 IU/ml, 1 ml at 10^4 IU/ml, 1 ml at 10^5 IU/ml, and finally 1 ml of 10^6 IU/ml if good tolerance was established at the previous doses. Similarly, AX or AMP was given by the oral route at the following doses: 5, 50, 100, 250, and 500 mg. Tests were all done with a minimum 30-min interval between each.

Implementation of the protocol

Subjects were considered to be allergic if they developed an immediate skin test response, by either the prick or intradermal method, to at least one of the four haptens tested, had a negative skin test but positive RAST, or, if both skin test and RAST were negative, were positive after controlled administration of the drug.

To establish allergy to classical penicillin determinants or verify the existence of selective allergy to side-chain-specific determinants, we performed tests at different times, as shown in Fig. 1, with at least 1 week between evaluations. Subjects reporting an immediate reaction to a penicillin derivative who were skin test negative to BPO and MDM, RAST negative to BPO-PLL, and tolerated the maximum concentration of BP by the parenteral route were considered to have good tolerance to common determinants of BP. If a positive result was seen after any of the tests, the subjects were considered to be allergic. The next evaluation was with AX and AMP; if all results were negative, subjects were classified as nonallergic, but if at least one of the tests proved positive, they were classified as selective reactors. If the penicillin involved was different from those previously mentioned, and skin test and challenge were negative to all determinants described above (e.g., cloxacillin or penicillin V), a third evaluation with the culprit drug was made at a different time.

Results

After testing, 290 subjects were considered to have had an immediate reaction to a penicillin derivative. On the basis of the patient's own description, 205 (71%) had developed anaphylaxis and 85 (29%) urticaria and/or angioedema. This indicates that for every case of urticaria there were more than two cases of anaphylaxis. According to clinical history, the involvement of the different penicillins over the whole study period was as follows: AX in 64.8% of cases, followed by "unrecalled penicillin" in 15%, AMP in 13%, other penicillins in 3.8%, and BP in 2.8% of cases. The term "unrecalled penicillin" was used when the patient claimed to be allergic to penicillin but was unable to specify exactly which one. The involvement of the different penicillins, divided into five 2-year periods, showed that there was a tendency for AX involvement to increase compared to the others, rising from 50% in the period 1985/6 to 77.7% in 1993/4. On the other hand, AMP and BP fell from 20% to 5.7% and from 6.2% to 1.6%, respectively, over the same periods,

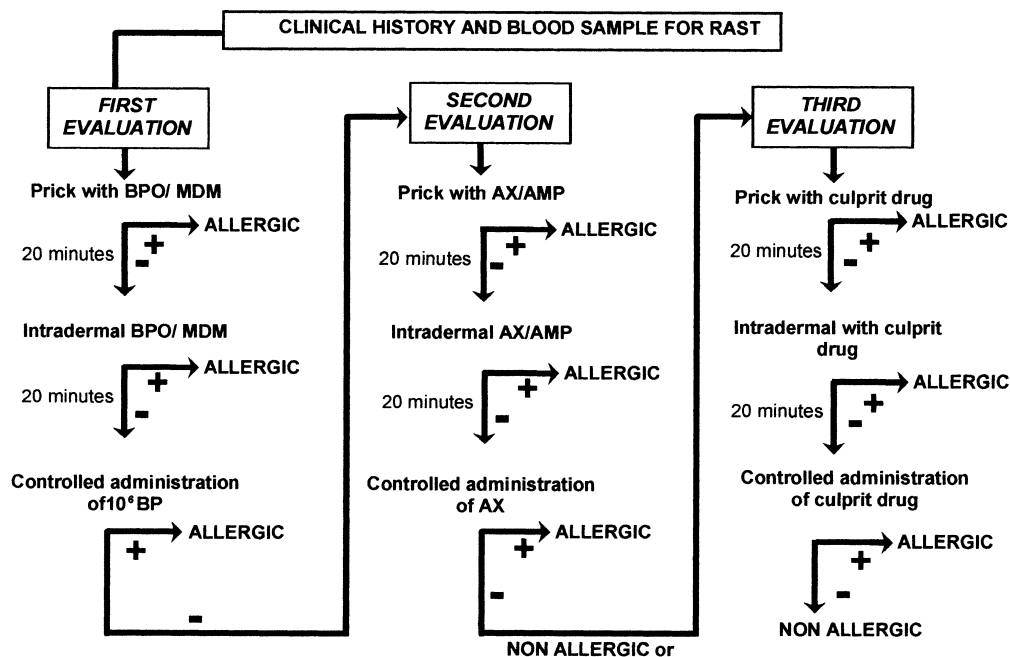


Figure 1. General approach in diagnosis of immediate reactions to penicillin derivatives. On left are shown all tests carried out with BP determinants, in middle those performed with AX and AMP determinants, and on right those with culprit drug. First and second evaluations were made in all cases studied, and third only in patients with selective reactions to other β -lactams.

almost a fourfold decrease. The other penicillins were cloxacillin in three cases, penicillin V in seven cases, and metampicillin in one case.

Of the whole group, 203 subjects (70%) had a positive skin test to at least one of the haptens tested (Fig. 2). In 54.7% of cases, the patients were positive to one or more determinants generated by BP (BPO and/or MDM), and 44.8% were positive only to AX and/or AMP determinants. Only one patient (1.4%) was skin test positive to AMP and negative to the other determinants, although he developed a reaction after administration of BP.

Systemic symptoms induced by skin testing occurred in 32 patients (11%), AX being responsible in 16 cases (50%), followed by BPO in nine (29%), MDM in five

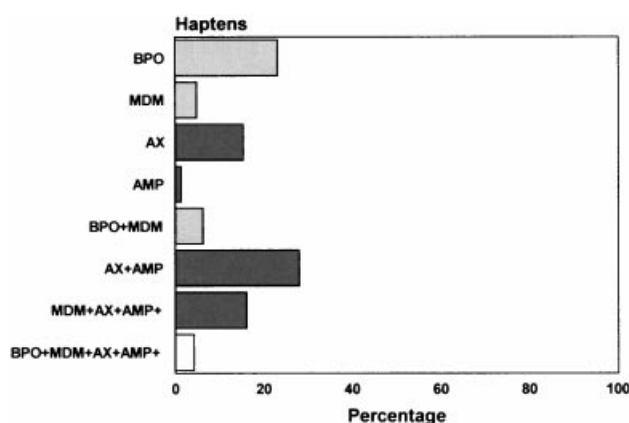


Figure 2. Percentage of skin test positivity to individual or combined haptens in group of skin-test-positive patients.

Table 1. Systemic symptoms induced by skin testing. Those in bold reacted by prick

Patient no.	Determinant	Dose	Clinical characteristics
1	AX	2 mg/ml	Systemic pruritus
2	MDM	2×10^{-2} mM	Erythema and conjunctivitis
3	MDM	2×10^{-2} mM	Urticaria, pruritus, and hypotension
4	BPO	5×10^{-5} mM	Urticaria
5	AX	20 mg/ml	Urticaria and lip angioedema
6	MDM	2×10^{-2} mM	Erythema and conjunctivitis
7	AX	2 mg/ml	Erythema, conjunctivitis, and dyspnea
8	AX	20 mg/ml	Urticaria and facial angioedema
9	BPO	5×10^{-5} mM	Urticaria, pruritus, and hypotension
10	MDM	2×10^{-2} mM	Nausea, pruritus, and hypotension
11	AX	20 mg/ml	Erythema and conjunctivitis
12	AX	2 mg/ml	Urticaria, dyspnea, and nausea
13	BPO	5×10^{-5} mM	Urticaria
14	AX	20 mg/ml	Generalized erythema and dyspnea
15	MDM	2×10^{-2} mM	Urticaria and nausea
16	BPO	5×10^{-5} mM	Erythema, conjunctivitis, and dyspnea
17	AX	2 mg/ml	Urticaria and conjunctivitis
18	AX	20 mg/ml	Systemic pruritus
19	BPO	5×10^{-5} mM	Generalized erythema and dyspnea
20	AX	20 mg/ml	Urticaria and abdominal cramps
21	BPO	5×10^{-5} mM	Erythema, conjunctivitis, and nausea
22	AX	20 mg/ml	Urticaria, hoarseness, and conjunctivitis
23	AX	20 mg/ml	Generalized erythema and dyspnea
24	AX	20 mg/ml	Urticaria, dyspnea, and nausea
25	AMP	20 mg/ml	Erythema, conjunctivitis, and dyspnea
26	BPO	5×10^{-5} mM	Generalized erythema and dyspnea
27	AX	2 mg/ml	Urticaria and hoarseness
28	BPO	5×10^{-5} mM	Urticaria
29	BPO	5×10^{-5} mM	Generalized erythema and hoarseness
30	AMP	20 mg/ml	Erythema, conjunctivitis, and nausea
31	AX	20 mg/ml	Urticaria and abdominal cramps
32	AX	20 mg/ml	Urticaria and nausea

Table 2. Positive responses to various penicillin determinants used according to prick or intradermal tests at different dilutions. Maximum possible concentrations used were BPO at 5×10^{-5} mmol/l, MDM at 2×10^{-2} mmol/l, and AX and AMP at 20 mg/ml

	BPO	MDM	AX	AMP
Prick*	21 (32.8%)	18 (29.5%)	48 (38.4%)	29 (30.5%)
ID*	37 (57.8%)	37 (60.7%)	62 (49.6%)	53 (55.8%)
ID (1/10)	5 (7.8%)	6 (9.8%)	14 (11.2%)	9 (9.5%)
ID (1/100)	1 (1.5%)	0	1 (0.8%)	4 (4.2%)
Total	64	61	125	95

*Skin test performed at maximum concentration.

ID: intradermal; BPO: benzylpenicilloyl; MDM: minor determinant mixture; AX: amoxicillin; AMP: ampicillin.

(15%), and AMP in 2 (6%). The reactions were anaphylaxis in 78% of cases, although in most instances they were mild, with urticaria in 15% and systemic pruritus in 6% of cases (Table 1).

The percentage of skin test positivity after prick or intradermal test is presented in Table 2. Prick test positivity to BPO appeared in 32.8% of the cases, and in a very similar proportion to the other determinants tested (MDM, AX, and AMP). The intradermal concentration inducing most positivity was the highest, with the lower dilutions barely contributing.

With regard to the *in vitro* studies, 13.1% of the patients were RAST positive but skin test negative, and the remaining 16.9% required a controlled administration for confirmation of the diagnosis. This 30% represents those cases which would have been missed if only the skin test had been used.

According to the criteria described above for classifying responders as nonselective or selective (*selective*: skin test negative to BPO and MDM, RAST negative to BPO-PLL, good tolerance to BP by the parenteral route, and positive results to any tests with AX and/or AMP; *nonselective*: positive results with any of the tests of penicillin determinants), 168 patients (57.9%) were diagnosed as positive to classical penicillin determinants and 122 (42.1%) to side-chain-specific determinants. Of these 122, almost all were positive to AX except one reacting to penicillin V and another to cloxacillin. No selective reaction to AMP was detected. The percentage of positivity after each of the three diagnostic procedures, divided into selective or non-selective responders, was different (Table 3). In the nonselective group, almost 75% of cases were diagnosed by skin testing, but in the selective group, this percentage decreased to 64% and controlled administration was necessary in almost 25% to establish the diagnosis. Comparison of these percentages by chi-square analysis, however, showed no statistically significant differences. The mean time interval between reaction and skin test procedure was 1214 ± 2093 days in the nonselective group and 268 ± 561 in the selective group ($P < 0.001$).

Since both previous experience (13, 21–23) and our experience indicate that patients with a positive history

Table 3. Results of positivity of different diagnostic tests in two groups: classical responders to BP (nonselective) and selective responders to AX

Group	Skin tests	RAST	Controlled	Total
Nonselective	125 (74.4%)	23 (13.7%)	20 (11.9%)	168
Selective	78 (63.9%)	15 (12.3%)	29 (23.8%)	122

plus positive skin test are highly likely to develop a reaction, and the risk of an acute allergic reaction is estimated to range from 50% to 70% in history-positive subjects, we did not give penicillin to these subjects to assess sensitivity because of the risk of inducing severe or uncontrolled reactions. Thus, we did not use controlled administration as the reference standard for classifying subjects as allergic or not allergic, but rather the criteria described in the Material and methods section. However, to establish specificity, we administered penicillins to subjects with no history of allergy and with known good tolerance to β -lactams. According to these data, we present in Table 4 the sensitivity (top) and the specificity (bottom) of skin testing. If we consider the contribution of any single hapten to a positive response, the sensitivity to BPO was 22%, to MDM 21%, and to AMP 33%, and the highest was 43% to AX. Since, in most instances, subjects tended to be skin test positive to more than one penicillin determinant, the combination of all four haptens gave a sensitivity of 70%. When we consider specificity, it can be seen that for each individual hapten figures range from 98% to 99%, decreasing to 97% when all the haptens are taken together. All control patients tolerated the administration of a penicillin derivative, although four were skin test false positive (Table 4).

Discussion

Immediate allergic reactions to β -lactams can be evaluated by different methods: clinical history, skin

Table 4. Sensitivity in positive group (on top) and specificity in negative group (on bottom). Positive group comprised all those who had positive history plus positivity in at least skin test, *in vitro* tests, or controlled administration

Hapten	Positive	False negative	Sensitivity	Total
BPO/MDM/AX/AMP	203	87	70%	290
BPO	64	226	22%	290
MDM	61	229	21%	290
AX	125	165	43%	290
AMP	95	195	33%	290
Hapten	Negative	False positive	Specificity	Total
BPO/MDM/AX/AMP	116	4	97%	120
BPO	119	1	99%	120
MDM	117	3	98%	120
AX	119	1	99%	120
AMP	119	1	99%	120

BPO: benzylpenicilloyl; MDM: minor determinant mixture; AX: amoxicillin; AMP: ampicillin.

tests, *in vitro* quantification of IgE antibodies, and controlled administration of the drug. The first three have generally been considered to be sufficient to confirm the diagnosis (5). However, since the sensitivity of these tests is not optimal, even with a clearly positive history, a controlled administration of the drug, which often results in good tolerance, may be required to establish the diagnosis. Although the clinical history is the starting point for the evaluation of patients allergic to β -lactams, this is not always reliable and additional tests are sometimes required. Skin tests with major and minor determinants of BP have been widely used (5, 25) and are considered almost mandatory unless serious contraindications exist (23, 26, 27). The *in vitro* test, although it has a lower sensitivity than the skin test, is in some instances important in confirming the diagnosis in patients negative in the skin test (28).

Initially, skin tests with BPO were considered to be positive in more than 70% of patients with IgE-mediated reactions to penicillin (5, 23, 24), although it was also known that a number of subjects were negative to BPO and responded to other penicillin metabolites that were defined as minor determinants (26). Most studies carried out over the years, mainly in the USA, have shown that BPO is the most relevant determinant, and that, combined with MDM, it is adequate to evaluate patients with immediate allergic reactions to β -lactams (5, 27–29). The appearance of semisynthetic penicillins led to the distinction of cross-reactivity from selective reactions (29, 30), with initial evidence indicating that the side chain plays little role in the specificity of the immunologic response, although differences in immunogenicity were observed (31). However, a few studies showed that semisynthetic penicillins could also induce skin test positivity (13, 32). The first consistent evidence of specific responses to penicillins was found in the Scandinavian countries in data from *in vitro* IgE determinations (33). However, since skin tests and controlled administration were not carried out, the significance of these *in vitro* findings was never established. The report of specific side-chain reactions in Spain in the late 1980s (18) and the 1990s (10), with confirmation by others in and outside Spain (34, 35), led to a more certain acceptance of this type of response.

We have been able to study a large population of patients with immediate allergic reactions to β -lactams over a long period of time. In the first 64 cases (10), we observed that the contribution of skin tests to positivity was 34.3% for BPO, 21.8% for MDM, 12.5% for AX, and 3.1% for AMP. The present report of 290 cases detected over a period of 10 years, including those described in our first observation, showed that positivity to BPO appeared in 22% of cases, to MDM in 21%,

to AX in 43%, and to AMP in 33%. Moreover, in the present study, skin tests were positive in only 70.3% of the cases. Although no studies have been carried out in Spain in the same population by the same group, data reported in 1979 indicated that the most relevant determinant was BPO, with AMP and BP being the drugs most commonly prescribed (36). Although different reports have indicated that other semisynthetic penicillins such as cloxacillin, metampicillin, or ampicillin could contribute to positive skin test responses (13, 29), detailed analyses in both Spanish and Italian populations have shown that AMP does not induce specific reactions (11).

This study makes two observations of note. The first is the predominance of anaphylaxis over urticaria, which may explain why minor determinants are more frequently positive than BPO (5, 27, 38); the second is the number of cases in which a controlled administration was required to confirm the diagnosis, contrasting with previous data indicating that in subjects with negative skin test to BPO and MDM the risk of having a reaction after penicillin administration is negligible (5, 23). However, at present, even including AX and AMP or other determinants in the skin test, the population that can be diagnosed is still lower than before. We can therefore conclude that skin test sensitivity is now lower than previously estimated (34, 36, 39). It is also relevant to mention that, although prick tests detected positivity in 30% of the cases, in intradermal tests the most sensitive concentration was the maximum used. Similar data have been reported by other authors (15).

The appearance of systemic reactions after skin testing involving minor determinants in 71% of cases and BPO in 29% is a matter of concern. This suggests that since anaphylaxis seems to be a more common manifestation nowadays, more precaution than previously recommended should be taken when carrying out skin tests (5).

In conclusion, immediate allergy to β -lactams is still a relevant problem and one that is becoming more complex due to the wide variety of β -lactams available and to the decrease in the sensitivity of skin testing. Although this is the largest study to date on immediate allergy to β -lactams, the findings need to be contrasted with those in other countries because of the variability in the response that can be found among different populations.

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